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Biofeedback-Assisted Stress Management Training to Reverse Myocardial Remodeling in Patients with End-Stage Heart Failure

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BIOFEEDBACK-ASSISTED STRESS MANAGEMENT TRAINING TO REVERSE MYOCARDIAL REMODELING IN PATIENTS WITH END-STAGE HEART FAILURE

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ABSTRACT

Heart failure is a progressive disease in which the heart is no longer able to pump sufficient amounts of blood to the body. Over six million Americans currently suffer from heart failure, and although pharmacological and surgical therapies continue to improve, about 50% of people with heart failure still die within five years of diagnosis.

As the human heart fails, many structural, cellular and molecular alterations occur that contribute to the decrease in heart function. It has been well-established that some of these alterations are the result of sympathetic nervous system hyperactivation, and decreasing sympathetic input with a beta blocker or left ventricular assist device improves clinical status and also reverses the cellular and molecular alterations associated with heart failure. We hypothesized that heart failure patients could be trained with biofeedback and that this method of sympathetic nervous system regulation would also produce myocardial remodeling in the direction of recovery.

To test this hypothesis, twenty end-stage heart failure patients listed for heart transplantation at the Cleveland Clinic received eight sessions of biofeedback-assisted stress management training. After biofeedback training, at the time of heart transplantation, explanted hearts were transported to the laboratory to study the heart failure phenotype. Data were compared to samples of non-failing, failing (negative control), and LVAD-supported failing (positive control) hearts.

We found that the inotropic response of left ventricular trabecular muscles to sympathetic nervous stimulation recovered in patients who received biofeedback training such that it was not significantly different from the non-failing average. Normalization of both rate of contraction and relaxation were also shown in the biofeedback group. Beta adrenergic receptor density was significantly lower in the biofeedback group relative to non-failing hearts, however significant recovery was shown in some patients. Western Blot analysis of calcium cycling proteins showed that SERCA and NCX expression in the biofeedback group was at the same level as the non-failing group, and a significant decrease in RYR expression was shown in the biofeedback hearts. These data suggest that biofeedback produces some remodeling of the heart failure phenotype, in the direction of non-failure.

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CHAPTER I

INTRODUCTION

Cardiovascular disease is the number one killer of men and women in the United States today, accounting for approximately 55% of all deaths.[1] Nearly 83 million Americans have one or more types of cardiovascular disease, including high blood pressure, coronary artery disease, angina pectoris, stroke, or congenital cardiovascular defects.[1] Although each form of cardiovascular disease represents a different etiology, the end-stage of all cardiovascular diseases is heart failure.

Heart failure occurs when the heart is no longer able to adequately pump blood to the body. While pharmacological therapies such as beta blockers and angiotensin converting enzyme inhibitors have been proven to alleviate the symptoms of this disease, heart failure continues to progress in patients receiving optimal pharmacological treatment,[75] and therefore heart transplantation is still the only option for long-term success.[94] Unfortunately, the number of donor hearts is limited, leveling off at about 2,300 per year,[117] and therefore many patients will succumb to the disease before a donor heart becomes available. Because of this, it is necessary to explore alternative or

adjunctive therapies to ameliorate disease progression or to provide a positive means for patients to cope with the disease sequelae. This project investigated the efficacy of biofeedback-assisted stress management training to reverse myocardial remodeling in patients with end-stage heart failure.

1.1 Normal Cardiac Physiology

Before introducing how the heart remodels during failure, it is important to note how the heart contracts in a normal, non-diseased state.

Excitation-Contraction Coupling and Calcium Cycling

Excitation-contraction coupling is a series of biochemical processes that converts electrical signals into mechanical signals through elevation of intracellular calcium.[9] Electrical activity is initiated when a specialized bundle of cells in the upper part of the right atrium of the heart, called the sinoatrial node, initiates a wave of depolarization that is propagated throughout the myocardium in an organized sequence, ultimately reaching the atrial and ventricular myocytes. This cardiac action potential depolarizes the myocyte sarcolemma, allowing calcium to enter the cell through L-type calcium channels. This small inward flux of calcium triggers a much larger release of calcium from inside the cell in a process called "calcium-induced calcium release." Specifically, the sarcoendoplasmic reticulum (SR), the main site of calcium storage in the cell, releases calcium through a calcium-sensitive channel known as the ryanodine receptor (RYR). This large release of calcium into the cytosol binds to the myofilaments and activates actin-myosin cross-bridging and cardiac muscle contraction.[9,8,49]

Because an increase in intracellular calcium is necessary for cardiac muscle to contract, there must be a decline in intracellular calcium concentration in order for cardiac muscle to relax. In cardiomyocyte relaxation, calcium is released from the myofilaments and is either pumped into the SR or extracted from the cell. Most free intracellular calcium (about 80%) is taken back up into the SR via the sarcoendoplasmic reticulum calcium ATP-ase (SERCA). Under baseline conditions, SERCA activity is partially inhibited by a calcium regulatory protein called phospholamban (PLB). When increased cardiac contractility is needed (such as during exercise or other sympathetic activation), PLB is phosphorylated and dissociates from SERCA, removing its inhibition and allowing SERCA to more rapidly remove calcium from the cytosol. For every molecule of ATP consumed, SERCA pumps two calcium back into the SR. Once inside the SR, calcium binds to calsequestrin (CALQ), one of several calcium-binding proteins that holds calcium in the SR until the next contraction. The other 20% of intracellular calcium is removed from the cell via the sodium-calcium exchanger (NCX). The NCX is an antiporter that brings three sodium into the cell for every calcium moved out into the extracellular space. SERCA and NCX are the two major systems involved in reducing intracellular calcium during cardiac muscle relaxation. **Figure 1** provides and overview of the calcium cycling process which occurs every time the heart beats.[5,6,96]

The Beta-Adrenergic Signaling Pathway

One of the major pathways existing in the heart that effects both heart rate and force of contraction through its effects on calcium cycling in the cardiac myocyte is the beta-adrenergic signaling pathway. Activation of beta-adrenergic signaling causes an

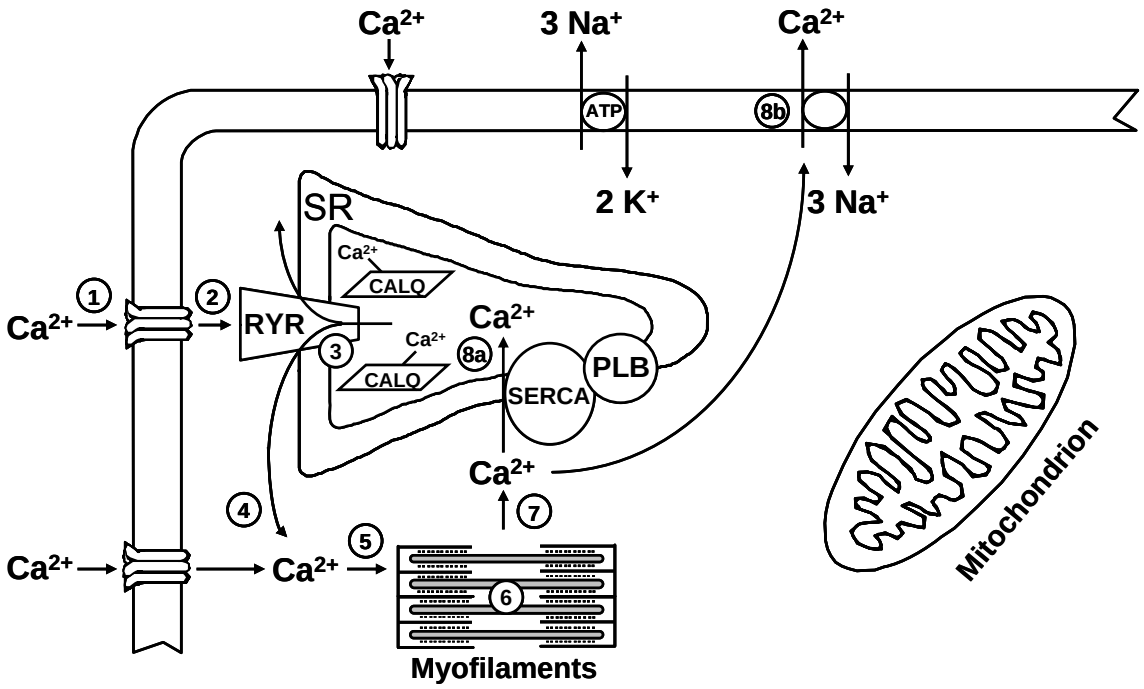


Figure 1. Calcium cycling in the cardiac myocyte.

Depolarization of the sarcolemma allows calcium to enter the cell through L-type calcium channels (1 & 2) which triggers the release of a large amount of calcium from the sarcoendoplasmic reticulum (SR) through the ryanodine receptor (RYR) (3) and into the cytosol (4 & 5). This increase in intracellular calcium binds to the myofilaments (6), causing cardiac muscle contraction. During relaxation, calcium is released from the myofilaments (7) and is pumped back into the SR via the sarcoendoplasmic reticulum calcium ATP-ase (SERCA) (8a) where it binds to calsequestrin (CALQ) until the next contraction. Some calcium is also extracted from the cell via that sodium-calcium exchanger (NCX) (8b). Phospholamban (PLB) regulates SERCA activity, inhibiting it in the baseline state.

(Figure 1 is modified from DM Bers, 1993.)[9]

increase in heart rate (chronotropy) and contractility (inotropy), as well as an increase in the speed of myocardial relaxation (lusitropy).[115]

The beta-adrenergic signaling pathway (illustrated in **Figure 2**) is activated when an agonist such as endogenous neurohormones epinephrine and norepinephrine, or isoproterenol (a synthetic analogue of norepinephrine), binds to the beta-adrenergic receptor on the surface of the cardiac myocyte. This causes activation of the stimulatory G protein (G_s) which is linked to the enzyme adenylyl cyclase (AC). Activation of AC then catalyzes the dephosphorylation of adenosine triphosphate (ATP) to form cyclic adenosine monophosphate (cAMP). Acting as a second messenger, cAMP activates protein kinase A (PKA), which phosphorylates many intracellular proteins including the L-type calcium channels, myosin binding protein C (MyBP-C), troponin I (TnI), phospholamban, and ryanodine receptors.[71,115]

Phosphorylation of the L-type calcium channels on the sarcolemma causes a greater influx of calcium into the cell during depolarization. By increasing the amount of intracellular calcium, more calcium is available to bind the myofilaments, and as a result, contractility is increased.[115,126]

Located at the level of the myofilaments, myosin binding protein C regulates the ATPase activity of the actin/myosin complex. Specifically, MyBP-C reduces ATPase activity, serving as a "brake" on crossbridge cycling. Phosphorylation of MyBP-C releases this brake, increasing the actin/myosin ATPase activity and enhancing cardiac contractility.[33,105,115]

Also located at the level of the myofilaments, Troponin I is one of the three regulatory proteins of the troponin complex. As the inhibitory subunit, phosphorylated

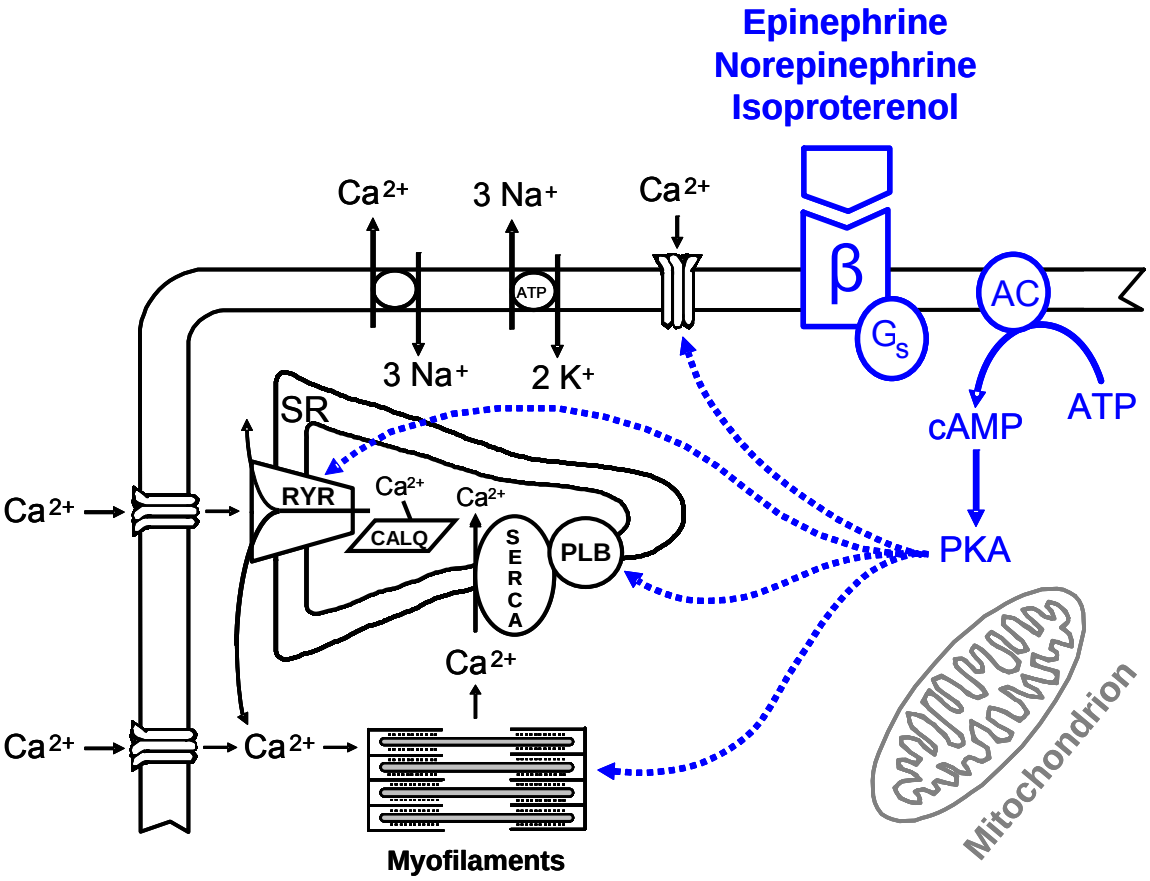


Figure 2. Beta-adrenergic signaling pathway in the cardiac myocyte.

Beta-adrenergic agonists bind to beta receptors on the surface of cardiac myocytes, initiating an intracellular signaling cascade resulting in the phosphorylation of L-type calcium channels, phospholamban (PLB), ryanodine receptors (RYR) as well as myosin binding protein C and troponin I which are located at the level of the myofilaments. Activation of this pathway results in an increased concentration of intracellular calcium, ultimately causing an increase in heart rate and force of contraction.

(Figure 2 is modified from DM Bers, 1993.)[9]

Troponin I decreases myofilament sensitivity to calcium, thereby increasing the rate at which calcium is released from the myofilaments. This free calcium, now in the cytosol, can be pumped into the sarcoendoplasmic reticulum (via SERCA) or extracted from the cell (via NCX), decreasing the amount of intracellular calcium and increasing the rate of myocardial relaxation.[105,115]

The rate of myocardial relaxation is also increased through phosphorylation of the regulatory protein phospholamban (PLB), located on the sarcoendoplasmic reticulum (SR). At baseline, PLB retards the speed at which the sarcoendoplasmic reticulum calcium ATP-ase (SERCA) pumps intracellular calcium into the SR. When PLB is phosphorylated, it releases this inhibition, and SERCA pumps calcium into the SR at a much faster rate. This decreases intracellular calcium concentration more quickly, increasing the rate of myocardial relaxation. In addition to increasing the rate of relaxation, phosphorylation of PLB also improves contractility. By allowing SERCA to pump faster, more calcium is taken up into the SR during relaxation, and therefore more calcium is stored in the SR for release during the next contraction.[105,115]

In addition to an increase in SR calcium storage, beta-adrenergic signaling also results in a greater amount of calcium being released from the SR into the cytosol through phosphorylation of ryanodine receptors. RYR phosphorylation causes these calcium release channels to be more sensitive to calcium-induced activation, releasing more calcium into the cytosol where it can bind to the myofilaments and increase cardiac contractility.[49,77]

The Muscarinic Signaling Pathways

Another set of pathways that exist in the heart to regulate heart rate and force of contraction are the muscarinic signaling pathways (**Figure 3**). Muscarinic signaling pathways are initiated when an agonist such as acetylcholine activates muscarinic receptors on the cardiomyocyte cell surface. Depending on which subtype of muscarinic receptor is activated, one of two different intracellular signaling cascades is initiated.[20,31]

In total there are five subtypes of muscarinic receptors (named M_1 – M_5 based on the order in which they were discovered) that signal through two different pathways. The even-numbered subtypes of muscarinic receptors (M_2 and M_4) directly antagonize the beta-adrenergic signaling pathway by inhibiting adenylyl cyclase through activation of the inhibitory G protein (G_i). This reduces intracellular cAMP concentration and inhibits PKA from phosphorylating its target proteins, effectively slowing down heart rate. In the presence of enhanced contractility (due to increased beta-adrenergic signaling), the M_2/M_4 signaling pathway can also cause negative inotropic effects in a phenomenon called "accentuated antagonism." [20,31,68,69]

Agonist activation of odd-numbered muscarinic receptors (M_1, M_3 and M_5) causes G_q to activate phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2), forming inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). DAG stimulates protein kinase C (PKC), and IP_3 travels to the SR where it is received by the IP_3 receptor, a calcium channel that releases calcium from the SR into the cytosol, increasing heart rate and force of contraction.[20,31]

1.2 Heart Failure: A Process of Remodeling

Heart failure is characterized by a process of cardiac remodeling involving

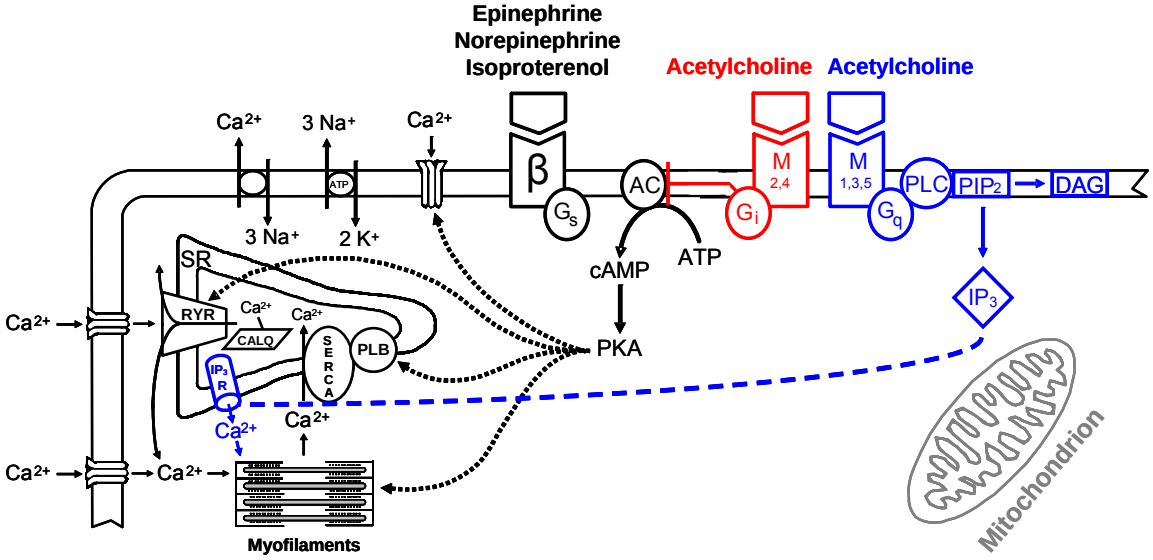


Figure 3. Muscarinic signaling pathways in the cardiac myocyte.

Muscarinic agonists bind to muscarinic receptors on the surface of cardiac myocytes, initiating one of two intracellular signaling cascades. Even-numbered muscarinic receptor subtypes, shown in red, oppose the beta-adrenergic signaling pathway by inhibiting adenylyl cyclase (AC), ultimately inhibiting the effects of PKA phosphorylation and decreasing heart rate. Odd-numbered muscarinic receptor subtypes, shown in blue, activate phospholipase C (PLC) which produces inositol 1,4,5-trisphosphate (IP_3) and ultimately causes the opening of a calcium channel in membrane of the sarcoendoplasmic reticulum (SR). Calcium flows into the cytosol from the SR, increasing intracellular calcium and myocardial contractility.

(Figure 3 is modified from DM Bers, 1993.)[9]

cellular and molecular changes that manifest clinically as changes in heart size, shape and function. As the human heart fails, it increases in size and mass due to an increase in individual cardiac myocyte length. Ventricular chambers dilate producing thinner walls, and the overall geometry of the heart shifts from an ellipse to a sphere.[23,24,43,101] The thin walls of the failing heart cannot normalize wall stress so contractile function declines. This decline in myocardial contractility emerges as a decrease in both left ventricular ejection fraction and cardiac output.[16]

In addition to the structural and functional alterations associated with the failing heart, there are also changes at the cellular and molecular level which have come to be known as the heart failure phenotype. These changes include activation of the immune system, as evidenced by the increased expression of inflammatory cytokines[76,109,112], a recapitulation of the fetal gene program[16], including ventricular expression of atrial natriuretic factor (ANF) and increased expression of both plasma ANF and B-type natriuretic peptide (BNP)[120], and a change in myosin heavy chain isoforms[72,85,88,99].

The heart failure phenotype also includes altered calcium homeostasis due to the differential regulation of various calcium cycling proteins. Specifically, SERCA mRNA[4,36,82], protein[36,52,83], and activity[48,52] decrease, causing lower calcium re-uptake into the sarcoendoplasmic reticulum (SR) and a much lower calcium-induced calcium release during the next depolarization. Phospholamban mRNA decreases, although it is uncertain if this is also true at the protein level.[36,83] Sodium-calcium exchanger mRNA[10,35,104], protein[10,35,53,54,98], and activity[10,53,98] are all upregulated in failing human hearts, increasing calcium extrusion from the cell. Consistently, no changes have been found with respect to calsequestrin mRNA[27,108] or protein[27,53,83] expression in failing

heart tissue.

Changes in beta-adrenergic signal transduction are also part of the heart failure phenotype. In heart failure, total beta-adrenergic receptor density is decreased, and beta-adrenergic responsiveness to catecholamines is also reduced.[18,19,27,38] As a result, myocardial contractility increases to a lesser degree following beta-adrenergic stimulation in failing human hearts as compared to non-failing hearts. In other words, the failing heart does not have the capacity to work harder when metabolic demands are placed on the body, such as during exercise[18]. This is the reason why the six minute walk test is conducted in heart failure patients as a marker of functional exercise capacity.

With all of these structural, cellular and molecular changes in mind, it is clear that the heart failure phenotype plays a pivotal role in the decreased contractility that is a hallmark of human heart failure.

1.3 The Autonomic Nervous System in Heart Failure

Another hallmark of heart failure is dysregulation of the autonomic nervous system. When the heart fails to pump blood effectively, inadequate tissue perfusion is sensed by the body, and the primary compensatory mechanism initiated to restore cardiac function is activation of the sympathetic nervous system (SNS). The SNS releases norepinephrine (NE) which increases heart rate and enhances contractility via the beta-adrenergic signaling pathway. While this is a successful short-term solution, chronic activation of the SNS is maladaptive and generally leads to pathophysiological processes such as arrhythmias, plaque rupture and myocardial cell death.[30,37,115] Sympathetic hyperactivity also leads to cardiotoxic levels of norepinephrine in the bloodstream, a

characteristic which has become a diagnostic of heart failure.[17,18,59,62] Many studies have shown a correlation between circulating levels of norepinephrine and worse prognosis in patients with heart failure.[22,25,40]

Overactivation of the SNS alone does not produce the entire autonomic dysregulation in heart failure. Sympathetic overactivity is accompanied by diminished parasympathetic nervous system (PNS) control of the heart.[13,29,93,96] This hypoactivation of the PNS can have pro-inflammatory consequences that exacerbate the heart failure condition[57,113], and some studies have even suggested that vagal withdrawal may be as deleterious as the overactive SNS.[13] Many studies have shown that restoring a normal balance of sympathetic and parasympathetic nervous system activation is associated with improved cardiovascular health.[13,39,69]

1.4 The Reversibility of Heart Failure

Until about fifteen years ago, heart failure was believed to be irreversible and amenable only to palliative care. Recent research, however, in patients who have been hemodynamically supported with a left ventricular assist device (LVAD), has shown that some of the cellular and molecular alterations associated with the failing heart can be reversed.

The LVAD is a mechanical pump that is surgically implanted into the abdomen of many advanced heart failure patients as a bridge to cardiac transplantation (**Figure 4**). With attachments at the apex of the left ventricle and the ascending aorta, the LVAD performs the function of the left ventricle, pumping blood to the rest of the body so that the ventricular muscle can rest. The patient's heart continues to beat, however it no

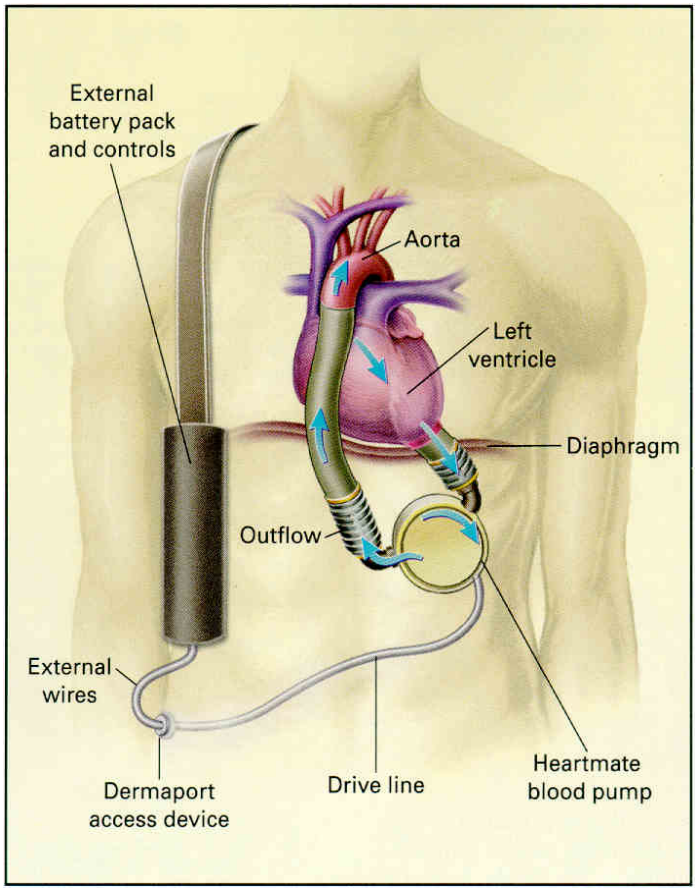


Figure 4. The left ventricular assist device.

The left ventricular assist device (LVAD) is a mechanical pump that is surgically implanted into the abdomen of advanced heart failure patients as a bridge to transplantation. Through its attachment to the apex of the left ventricle, blood enters the LVAD, and the LVAD pumps the blood into the circulation via its attachment to the ascending aorta.

(Figure 4 is from DJ Goldstein, 1998.)[44]

longer actively pumps blood, and it is therefore said to be unloaded.

Clinical studies of patients supported by an LVAD have shown improvements in both overall health and heart failure status[41,58,67]. LVAD support has also been shown to "reverse remodel" the failing heart by reversing many of the cellular and molecular alterations that are characteristic of the heart failure phenotype. Some of the alterations discovered in LVAD-supported heart failure patients include: a restoration of both whole heart[41,67,78,89] and individual myocyte size[2,28,67,89,125], improved contractile function[5,28,41,55,89], a decrease in circulating cytokines[112] and catecholamines[41,58,111] as well as decreased plasma ANF and BNP[2,122], a reversal of changes in gene expression[5,14,97], an increase in SERCA expression[5,55] as well as improvement in calcium cycling overall[5,28,55], an increase in beta receptor density[92] and responsiveness[5,28,92], and an inactivation of some of the signaling pathways leading to the disease[34,46]. Taken together, these findings illustrate that heart failure is not irreversible as was once believed.

1.5 Biofeedback

Biofeedback is a process through which individuals learn self-regulation skills that allow them to control their physiology for the purpose of improving health or performance. It involves specialized equipment to monitor one or more physiologic processes and convert the signals into meaningful visual and auditory cues that are fed back to the client in real time. Typically, sensors are connected to the client, measuring modalities such as heart rate, muscle tension and finger temperature, and the information is immediately displayed on a computer screen. These physiologic processes are

normally under control of the autonomic, or involuntary, nervous system. Biofeedback allows individuals to consciously control these involuntary physiologic processes, and in a clinical setting, this puts control over one's health and well-being into the hands of the patients themselves.[81,103]

While biofeedback can be used solely as operant conditioning, patients suffering from stress or a disease with a major stress component often benefit from using biofeedback in tandem with stress management.[66] This is because acute stress is mediated through the autonomic nervous system, and biofeedback trains patients to control autonomic activation. Specifically, psychological stress activates the sympathetic nervous system through what is well known as the "fight or flight" response. Biofeedback is therefore often used to decrease sympathetic nervous system input so that the parasympathetic nervous system can become more involved in regulating physiologic functions.[87]

When biofeedback is combined with stress management, it requires a trained biofeedback practitioner who does more than just explaining what the biofeedback equipment is and how it relates to the patient's physiology, as is done in the operant conditioning model.[81] Here, the biofeedback practitioner must also provide stress management techniques and relaxation skills such as guided imagery and progressive muscle relaxation. It also requires individualized training in which patients are evaluated for the specific vulnerabilities that lead them to hyperarousal. This is often achieved with a psychophysiologic assessment in which patients are shown how their body responds to mental stimuli. Baseline levels of physiologic modalities are measured before a series of mental stress tests and relaxation exercises. The biofeedback practitioner then guides the

patient through the thought and behavior patterns that contribute to their physiological vulnerability.[81,103] Once the mind-body connection is made, patients can differentiate between relaxed physiology and hyperarousal, knowing what each feels like. The ultimate goal of biofeedback, therefore, is to teach patients how to self-regulate so that they can calm their physiology when the biofeedback equipment is not in front of them.

Heart Rate Variability

Heart rate variability (HRV) is a physiologic modality that has come into use more recently as a measure of autonomic balance and cardiovascular resilience. HRV is a measure of the beat-to-beat fluctuations in heart rate, which like any other stable system in the body, must be able to change and adapt in order to maintain homeostasis.[66] The main inputs to the cardiovascular system that use feedback mechanisms to regulate heart rate are the autonomic nervous system, respiration rate, and the baroreceptors. These oscillations in the system allow it to respond to stress, disease and injury, much like a boxer shifts his or her stance in order to respond to the next punch.

The clinical relevance of HRV was first noticed in 1965 when a lack of HRV was observed prior to fetal distress.[56] We now know that decreased HRV is associated with many diseases and disorders involving autonomic nervous system dysfunction or stress including cardiovascular disease, depression, anxiety, etc.[60,66,116] Since then, the field has evolved, and in 1996, a task force was formed to determine standards of measurement, interpretation guidelines, and clinical applications of HRV.[110]

HRV can be measured in several ways, all beginning with an analysis of R waves on an electrocardiogram (EKG). While heart rate is calculated as the number of R waves

per minute, HRV is a measured of the amount of time between successive R waves (the inter-beat interval). Inter-beat interval (IBI) data are measured in milliseconds (msec) can be calculated from the heart rate by dividing 60 seconds by the heart rate (in beats per minute). The resulting value is in seconds, so multiplying this value by one thousand will give you the IBI value in msec (**Figure 5**). Because heart rate is typically reported as an average over time (beats per minute), it can be the same value with equal IBIs or with variable IBIs. Several studies have shown that variability in the IBI (heart rate variability) is a marker of prognosis in cardiovascular disease, namely that greater HRV is a predictor of greater cardiovascular health. It is believed that patients with end-stage heart failure have little or no variability in their heart rate.[12,61,90]

1.6 Summary and Hypothesis

As previously mentioned, (1) heart failure has structural and functional bio-markers that allow disease progression or recovery to be measured, (2) autonomic nervous system dysfunction is a hallmark of heart failure, with an increase in sympathetic activity and a withdrawal of parasympathetic control (3) the process of heart failure can be reversed through LVAD support, and (4) biofeedback is a tool that can be used to teach patients to alter autonomic input to the cardiovascular system.

We hypothesized that use of biofeedback-assisted stress management training to downregulate sympathetic nervous system activity and upregulate parasympathetic nervous system activity, will provide a non-invasive psychophysiologic means to reverse myocardial remodeling in patients with end-stage heart failure.

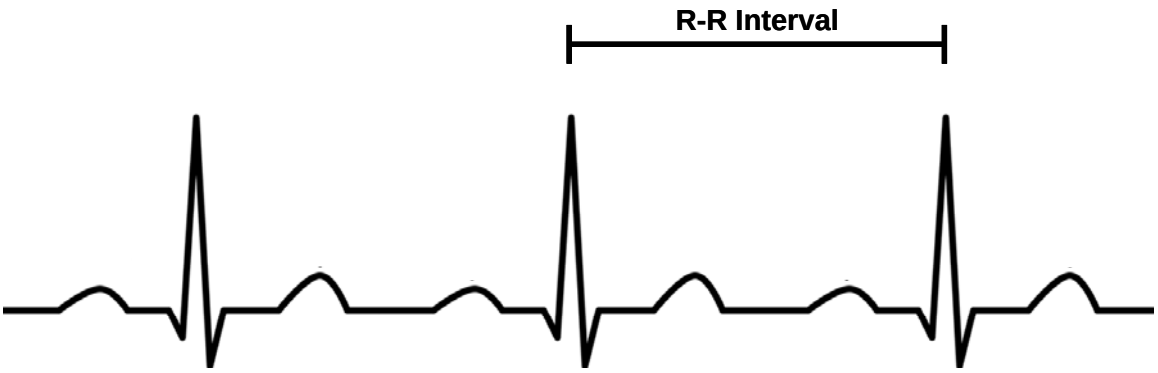


Figure 5. Heart rate variability.

Heart rate variability (HRV) is the beat-to-beat fluctuation in heart rate, also called the inter-beat interval (IBI). It has recently joined other biofeedback modalities as a direct measure of autonomic balance and cardiovascular resilience. Greater HRV has been associated with greater cardiovascular health, and patients with end-stage heart failure are believed to have no HRV.

CHAPTER II

METHODS

2.1 Patient Selection

Patients were enrolled in the experimental group (failing + BF) if they had advanced heart failure (New York Heart Association Class III or Class IV) and were listed for cardiac transplantation at the Cleveland Clinic. All patients received standard medical therapy for heart failure, including maximally tolerated doses of angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, aldosterone antagonists and cardiac resynchronization therapy / implantable cardioverter defibrillators if indicated by their heart failure cardiologist. Inpatients were also treated with intravenous inotropic agents.

Patients who were excluded from the failing + BF group include those who had a mechanical assist device at the time of transplant listing or those who required an assist device while waiting for transplantation. Patients were also excluded if they did not speak English and required a translator in order to communicate or if they were unable to return to the Cleveland Clinic for eight visits. Patients in the non-failing, failing and failing + LVAD groups were gender-, age-, race-, and (in the failing and failing + LVAD

groups) heart failure diagnosis-matched with those in the failing + BF group.

2.2 Biofeedback Protocol

All patients enrolled in the failing + BF group received the biofeedback-assisted stress management training, and each patient served as his or her own control. Patients were not randomized because it was unknown whether advanced heart failure patients would even be able to do biofeedback. Inpatients awaiting transplant in the hospital were studied in their rooms twice per week for 4 weeks. Outpatients awaiting transplant at home were studied in the Clinical Research Unit at the Cleveland Clinic once per week for 8 weeks. During training, patients were either seated comfortably in a recliner or lying supine in their hospital bed.

The biofeedback protocol was conducted by a biofeedback-certified psychologist in the presence of a biofeedback technician. The role of the technician was to hook the patient up to the biofeedback equipment at the beginning of each session and to monitor the raw data throughout each session, addressing any noise in the signal, if possible. Signal artifact as well as anything unusual that may have occurred during the session was noted by the technician. The technician was also present to confirm that the biofeedback therapist was using only biofeedback and stress management techniques with the patients. Specifically, it was important to have an independent party confirm that no psychotherapy was being conducted during the biofeedback sessions.

Sensors and Screens

Standard biofeedback equipment from Thought Technology (Montreal, QC) was used to monitor physiologic processes. Physiologic modalities measured included respiration

rate, digital peripheral temperature, skin conductance, heart rate, and heart rate variability in the time domain. **Figure 6** shows the four sensors that were used to monitor patients' physiology, and a summary of values when a person is relaxed is provided in **Table I**.

To measure respiration rate, patients were asked to exhale fully, and a strain gauge was placed around their waist, just above the belly button with a small amount of tension. The strain gauge is sensitive to stretch, and it converts the expansion and contraction of the patient's abdomen into a rise and fall in the signal transmitted to the computer. The software calculates respiration rate from this raw waveform (in breaths per minute).

Digital peripheral temperature was measured using a thermistor that was taped along the palmar side of the patient's fifth digit using Millipore medical tape. Changes in finger temperature were converted into an electrical current which was transmitted to the computer to display changes in the signal (in degrees Fahrenheit - °F).

To measure the skin's ability to conduct electricity, or skin conductance, silver-silver chloride electrodes embedded into Velcro straps were placed onto the pads of the patient's second and fourth digits. When in use, a small electrical voltage was applied through the electrodes, establishing an electrical current in which the patient serves as a resistor, and the real-time variation in conductance (the inverse of resistance) was recorded (in microsiemens - μS).

Lastly, heart rate was measured using a blood volume pulse sensor that was taped to the pad of the patient's third digit. This sensor uses photoplethysmography to shine infrared light through the skin and measure the light that is reflected back to the sensor. Because blood reflects red light and absorbs other colors, the amount of light reflected back to the sensor changes with the amount of blood flowing through the finger. The

Figure 6. Biofeedback sensors.

Strain gauge (A) used to measure respiration rate; Thermistor (B) used to measure finger temperature; Electrodes (C) used to measure skin conductance; Blood volume pulse sensor (D) used to measure heart rate / heart rate variability.

Photos taken from www.thoughttechnology.com/sensors.htm

Table I. Physiologic Signals When A Person Is Relaxed

RESP	DPT	SC	HR
6 breaths per minute	~ 90°F	2μS	~ 60-80 beats per minute

RESP = Respiration Rate, DPT = Digital Peripheral Temperature, SC = Skin Conductance, HR = Heart Rate

blood volume pulse signal is a relative measure from which heart rate (beats per minute) and inter-beat interval (milliseconds) are calculated. The raw inter-beat interval data were used to calculate measures of heart rate variability in the time domain.

Custom biofeedback screens were created to provide optimal audio and visual feedback to the patients in this study. As shown in **Figures 7-10**, each patient screen focused on a single physiologic modality. **Figure 11** illustrates a screen that was customized for the biofeedback therapist and the biofeedback technician to see a real-time summary of all physiologic modalities at the same time.

Session Outline

The first and last study visits (visits 1 and 8) consisted of a custom-designed 32-minute psychophysiologic assessment of stress reactivity. During this assessment patients were guided through three mental stress tasks, separated by five minute periods of rest / self-relaxation. A five-minute self-relaxation was also conducted at the beginning of each assessment, prior to the stressors. Mental stress tasks included the Stroop Color Word Test[74], Serial Sevens Test[102] and a Stressful Event Recall Task[103] in which patients were asked to talk about how it feels to be waiting for a heart transplant.

The six biofeedback-assisted stress management training sessions (visits 2-7) were each 45 minutes in length and involved basic relaxation training with biofeedback, predominantly respiration and temperature feedback. Although each training session began and ended with a 5-minute self-relaxation, the rest of the session consisted of individualized stress management exercises to which the patient best responded.

A schematic outline of all eight biofeedback sessions can be found in **Figure 12**.

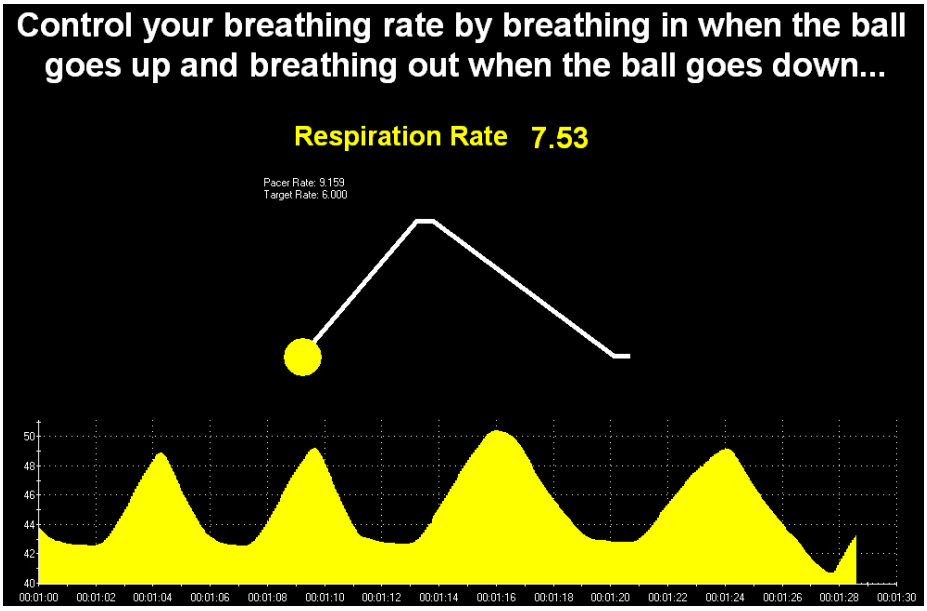


Figure 7. Respiration rate patient training screen.

The target breathing rate for patients was 6 breaths per minute. Using an adaptive pacer ball, patients were paced to breathe at a rate two breaths per minute less than their current respiratory rate, which was displayed digitally on the screen (in yellow). A filled line graph of respiratory amplitude was also displayed for patients to learn to breathe smoothly and deeply.

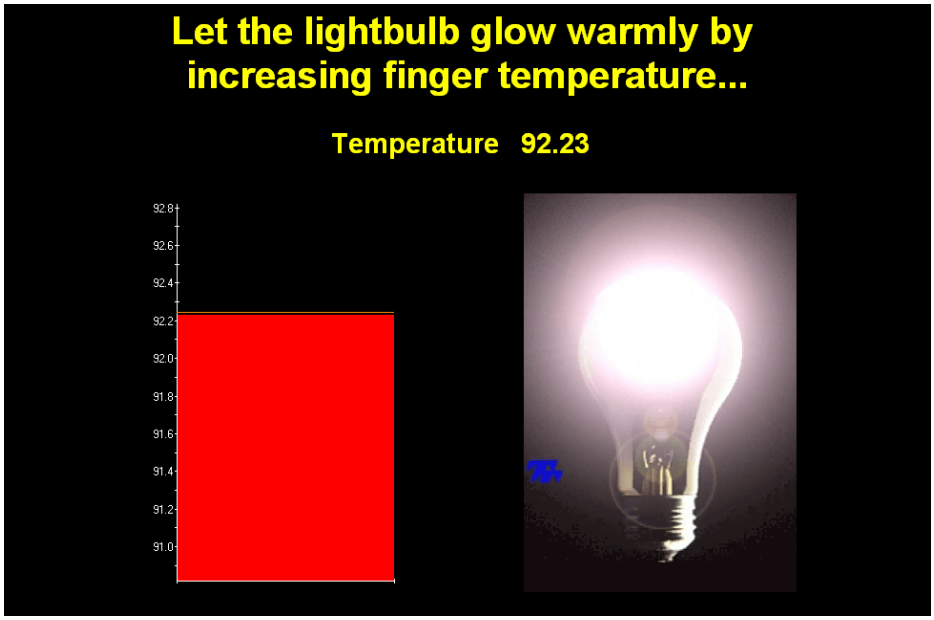


Figure 8. Digital peripheral temperature patient training screen.

In addition to a digital display of finger temperature (in yellow), this screen also includes a bar graph that is red as temperature rises and turns blue as temperature falls. The lightbulb is connected to the bar graph such that a rise in temperature lights up the bulb, and the bulb gets dimmer as finger temperature drops.

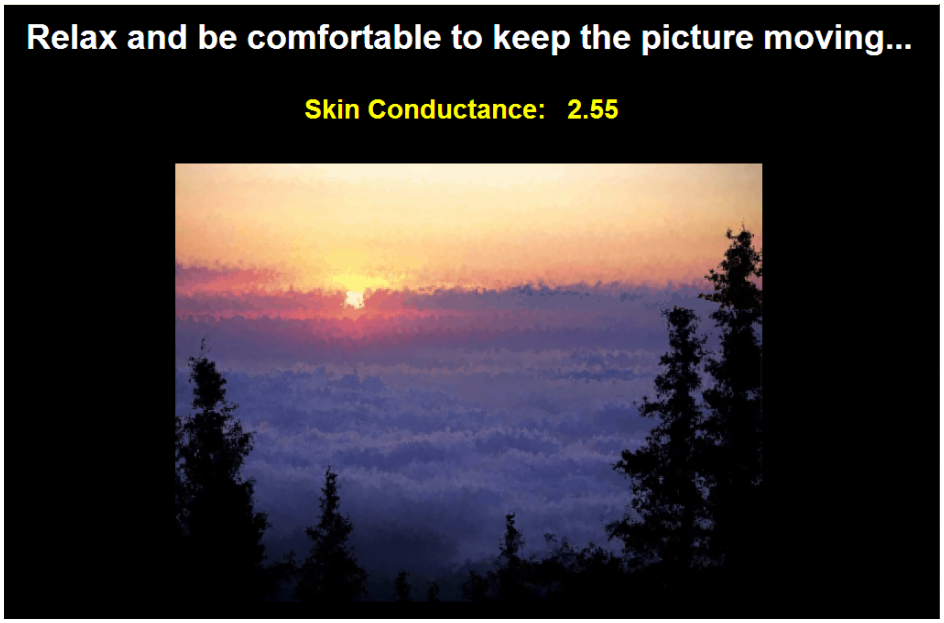


Figure 9. Skin conductance patient training screen.

This screen shows a slideshow of relaxing images that cycle as the patient lowers their skin conductance. If skin conductance rises, the current photo goes out of focus, and the slideshow does not advance. A digital display of skin conductance is also present on the screen (in yellow).

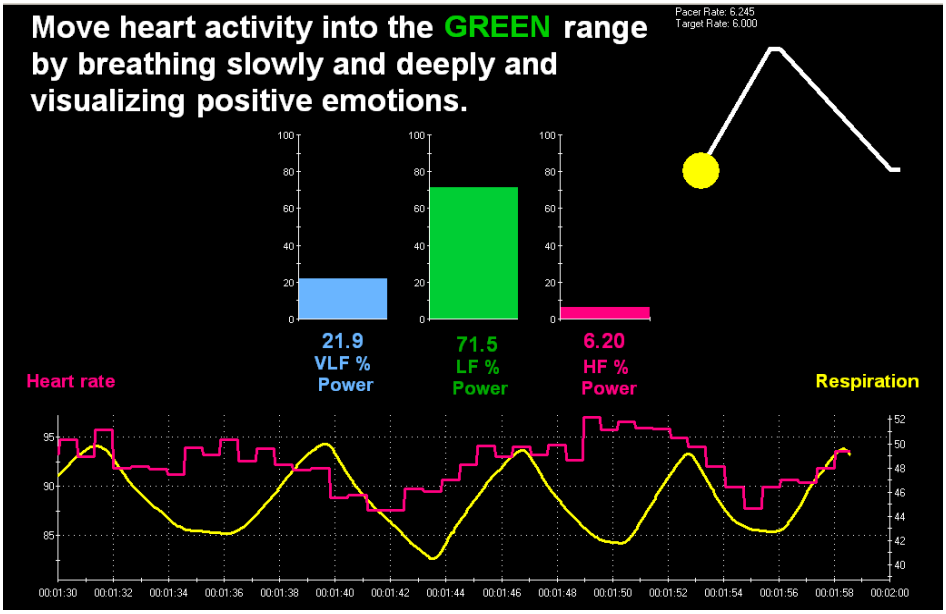


Figure 10. Heart rate variability patient training screen.

Patients were not formally trained using this HRV screen, but HRV was monitored throughout all sessions. This screen shows an adaptive pacer ball as well as a line graph displaying the overlap between heart rate and respiration rate. Bar graphs of HRV in the frequency domain are also displayed, however this screen was only ever casually referred to if the green bar was high so that the biofeedback therapist could provide positive reinforcement to the patient.

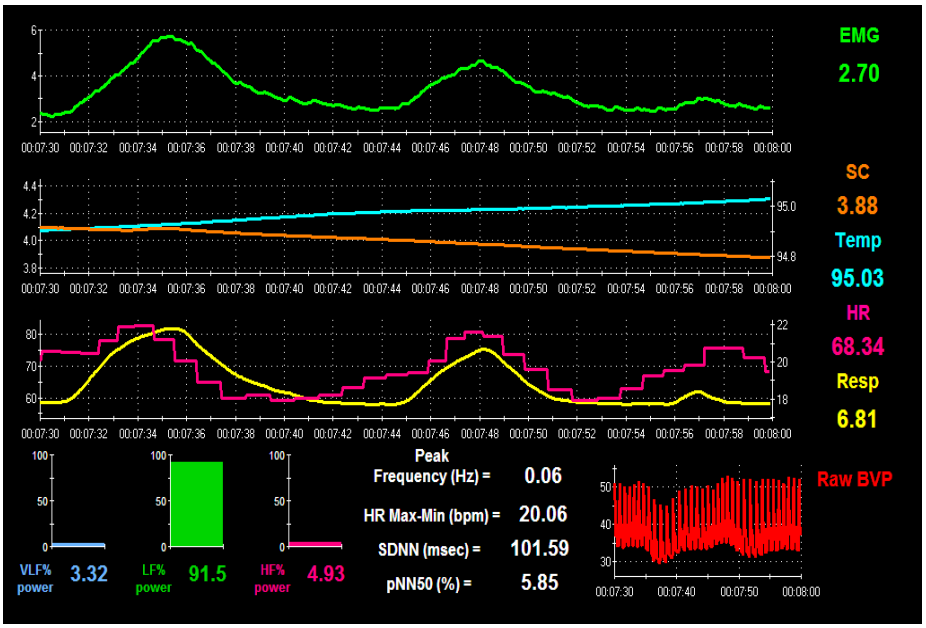


Figure 11. Biofeedback therapist / technician screen.

This screen was created so that all physiologic modalities being measured could be viewed simultaneously by the biofeedback technician and the biofeedback therapist.

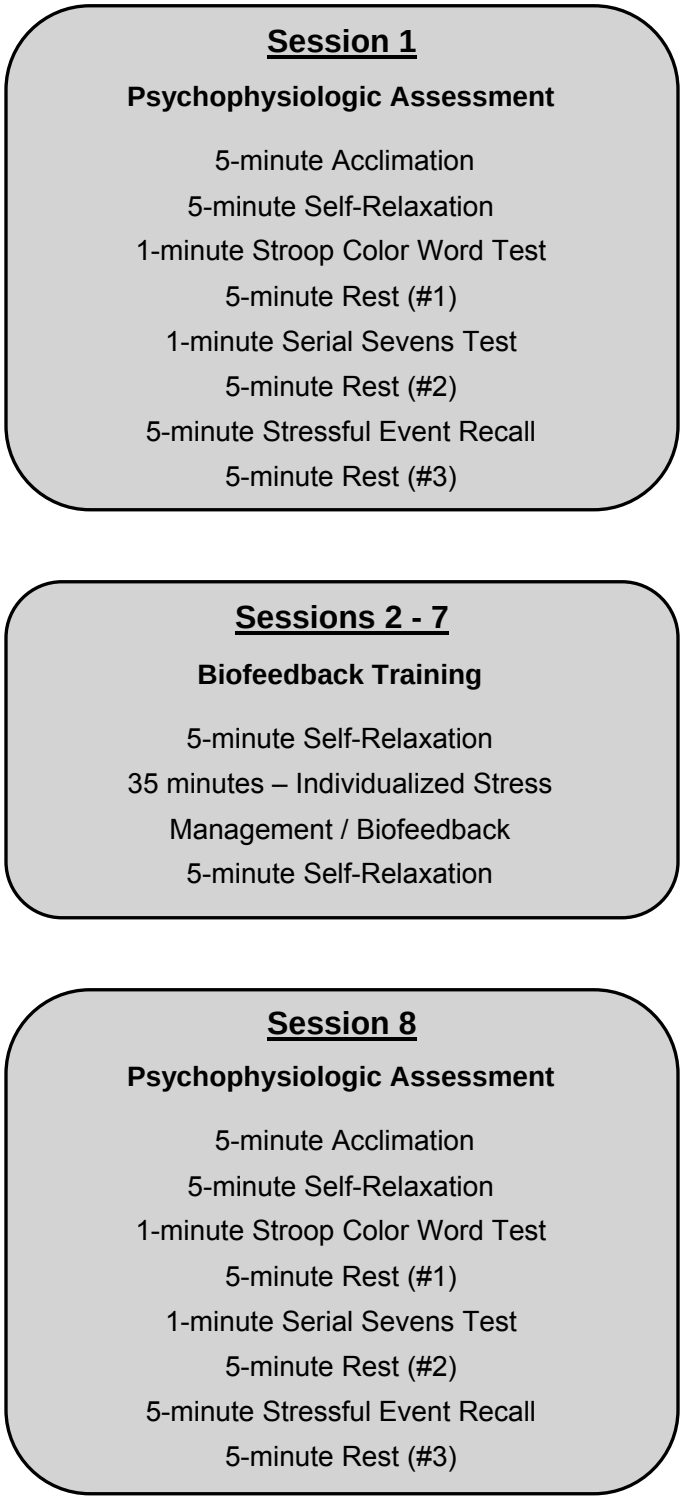


Figure 12. Schematic Outline of Biofeedback Sessions.

Homework

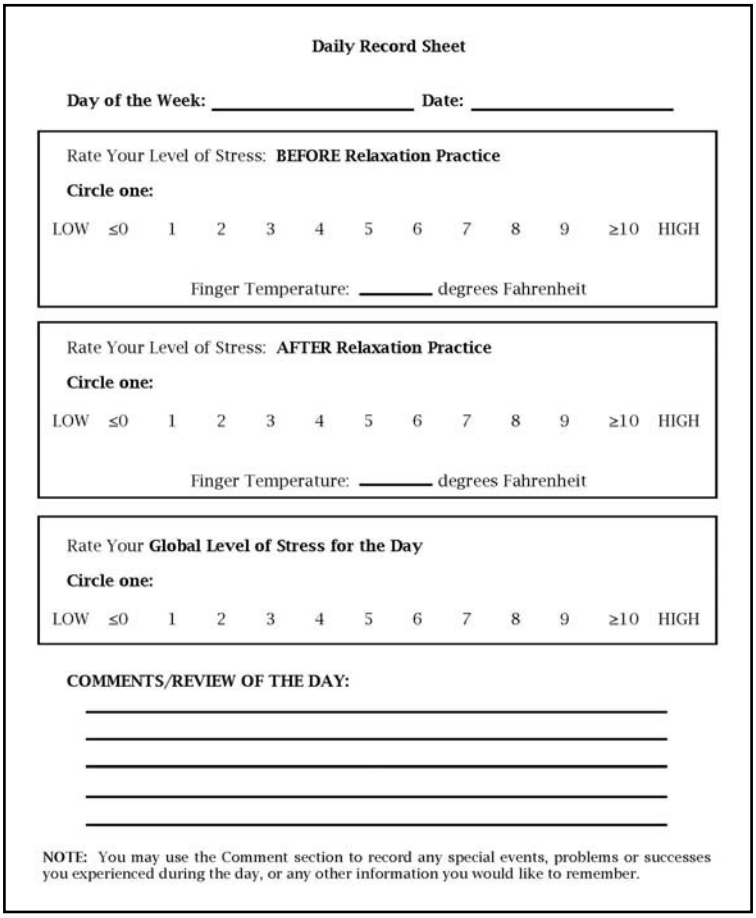
All patients were provided with relaxation CDs, handheld thermometers and daily record sheets. Patients were asked to practice at least 20 minutes per day on their own in between sessions, recording their daily stress levels and their finger temperature before and after relaxation practice. This home practice encouraged optimal learning of the biofeedback skills which were being taught in the training sessions. Daily record sheets (**Figure 13**) were collected at each visit, and patients were asked about the frequency and success of practice, which were also recorded.

Quality of Life Assessment

In order to assess whether biofeedback-assisted stress management enhanced quality of life for patients awaiting heart transplantation, two questionnaires were administered prior to the psychophysiologic assessment in the first and last biofeedback sessions. These included the RAND Short-Form 36 (SF-36) general health survey[79,80,119] and the heart failure-specific Kansas City Cardiomyopathy Questionnaire (KCCM)[47]. Both were scored according to standard metrics.[47,118]

Subjective Data

Patients were also asked several times throughout each biofeedback session to self-report aloud their heart failure status, level of relaxation and mood, and these responses were recorded. A 5-point Likert scale was used for each question with 1 being the most negative response and 5 being the most positive.



Daily Record Sheet

Day of the Week: _____ **Date:** _____

Rate Your Level of Stress: **BEFORE Relaxation Practice**

Circle one:

LOW ≤0 1 2 3 4 5 6 7 8 9 ≥10 HIGH

Finger Temperature: _____ degrees Fahrenheit

Rate Your Level of Stress: **AFTER Relaxation Practice**

Circle one:

LOW ≤0 1 2 3 4 5 6 7 8 9 ≥10 HIGH

Finger Temperature: _____ degrees Fahrenheit

Rate Your **Global Level of Stress for the Day**

Circle one:

LOW ≤0 1 2 3 4 5 6 7 8 9 ≥10 HIGH

COMMENTS/REVIEW OF THE DAY:

NOTE: You may use the Comment section to record any special events, problems or successes you experienced during the day, or any other information you would like to remember.

Figure 13. Daily record sheet.

Copies of this data sheet were provided to all patients, and they were encouraged to practice relaxation skills at home in between sessions, fill out these data sheets (one/day), and return them at their next visit.

Clinical Assessment

Clinical course was assessed in ambulatory outpatients during the first and last biofeedback sessions. Upon arrival, patients were asked to lay supine for 30 minutes before blood was drawn for measurement of plasma norepinephrine. Following the psychophysiologic assessment in these two sessions, a six minute walk test was performed for the objective evaluation of functional exercise capacity.[3]

2.3 Human Heart Tissue Procurement

Human heart tissue was acquired at the time of cardiac transplantation. Immediately after excision, each heart was obtained from the operating room and placed into chilled cardioplegia (concentration in mM: 77.0 NaCl, 20.0 KCl, 10.0 $NaHCO_3$, 14.0 Glucose, 0.1 $CaCl_2$) for transport to the laboratory. Non-failing human hearts were obtained through LifeBanc of Northeast Ohio from unmatched organ donors in which the heart was not suitable for transplantation due to histoincompatibility or a difference in organ size. Failing human hearts, including those who received biofeedback training and those supported by an LVAD, were obtained from recipients undergoing heart transplantation at the Cleveland Clinic. Informed consent was acquired prior to tissue procurement in accordance with an approved IRB protocol (IRB #2378, C Moravec Principal Investigator).

2.4 Cardiac Muscle Function

All muscle function experiments were performed at the time of transplant. Explanted hearts were brought back to the lab, and fresh left ventricular trabecular muscles were

immediately dissected from the endocardial surface of the heart. Individual muscles were placed between two O-rings and hung in a tissue bath filled with Krebs-Henseleit buffer (composition in nM: 100 NaCl, 4 potassium chloride (KCL), 1.5 magnesium sulfate heptahydrate ($MgSO_4$*7H2O), 20 sodium bicarbonate ($NaHCO_3$), 1.5 sodium phosphate (NaH_2PO_4), 20 sodium acetate, 10 glucose, 0.1 ascorbic acid, 2.5 anhydrous calcium chloride, 5 I.U. insulin) maintained at 37°C (**Figure 14**).

For the next hour, total muscle tension was monitored and adjusted every 15 minutes to ensure that it stayed close to 1.0g. No stimulus was given during this time. After the muscles stabilized, *GRASS* stimulators were turned on to provide repeat stimulation (10V, 1Hz), and the minimum voltage necessary to elicit muscle contraction (called the threshold voltage) was determined. To determine the threshold voltage, voltage was dialed back to 1V and steadily increased by 1 until the muscle began to contract. Voltage was then reduced by 1V (a voltage where the muscle did not contract) and slowly increased by 0.2V until the muscle contracted repeatedly (for ~ 30 seconds).

The stimulator for each muscle was set to a voltage 20% greater than the empirically determined threshold voltage, and this voltage was used for the remainder of the experiment. A length-tension curve was then performed to determine the length at which each muscle produces its greatest contraction (Lmax). With each muscle at its Lmax, the effect of any experimental manipulation can be compared across muscles. To find Lmax, baseline resting tension (RT) and developed tension (DT) were recorded, and after 1 minute, each muscle was stretched by 0.1mm. RT and DT were recorded 1 minute later, and the process continued (stretch muscles, wait 1 minute, record RT and DT) until there was a large increase in RT and DT no longer or barely increased. After Lmax was

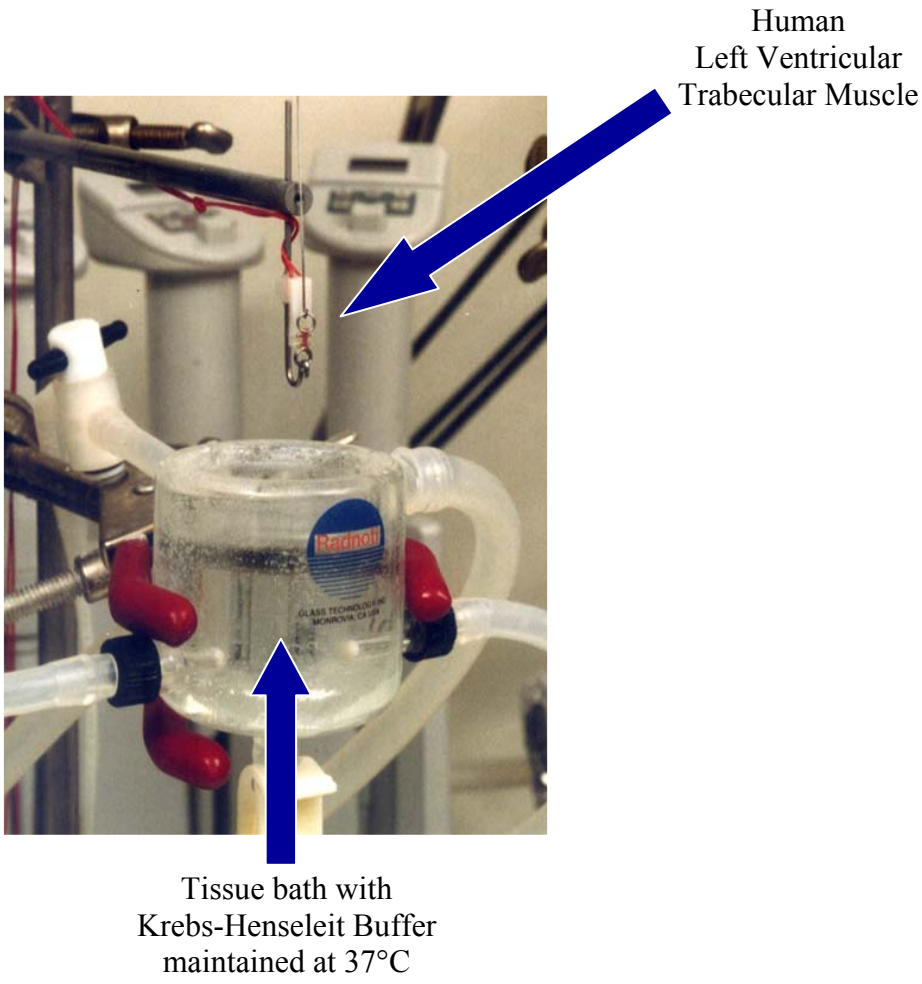


Figure 14. Muscle bath set-up.

Human left ventricular trabecular muscles are dissected from the endocardial surface of the heart and secured between two O-rings. Muscles are then hung in an oxygenated tissue bath containing Krebs-Henseleit buffer which is maintained at 37ºC.

reached, muscles stabilized in the tissue baths for 30 minutes, and only muscles with developed tension greater than 0.20g were used for the experiment.

Baseline data were collected for 5 minutes before 1μM isoproterenol (ISO), a laboratory analogue of norepinephrine, was added directly to each tissue bath to mimic stimulation by the sympathetic nervous system. After 10 minutes of data collection under the influence of ISO, data were saved in the data acquisition software (LabChart 7 Pro), and the tissue baths were lowered. Calipers were used to measure the length of each muscle between the O-rings, and then each muscle was carefully cut out as close to the O-rings as possible. Muscles were dried in bibulous paper for exactly 15 minutes and immediately weighed. The cross-sectional area (XSA; in mg/mm) of each muscle was calculated by dividing the weight of each muscle by its length.

Muscle function at baseline (Lmax) as well as the response to isoproterenol was analyzed for six contractile parameters including RT, DT, time to peak tension (TPT), time to half relaxation (THR), peak rate of tension rise (+dT/dt), and peak rate of tension fall (-dT/dt) (**Figure 15)**.

2.5 Sarcolemmal Membrane Isolation and Purification

In preparation for beta-adrenergic and muscarinic receptor density measurements, sarcolemmal membrane fragments were isolated and purified from frozen human heart tissue which had been stored at -80°C. Frozen tissue samples weighing 2.0g ± 0.2g were each placed into 20mL of chilled homogenization buffer A (composition: 10mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 5mM ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 12.5mM magnesium

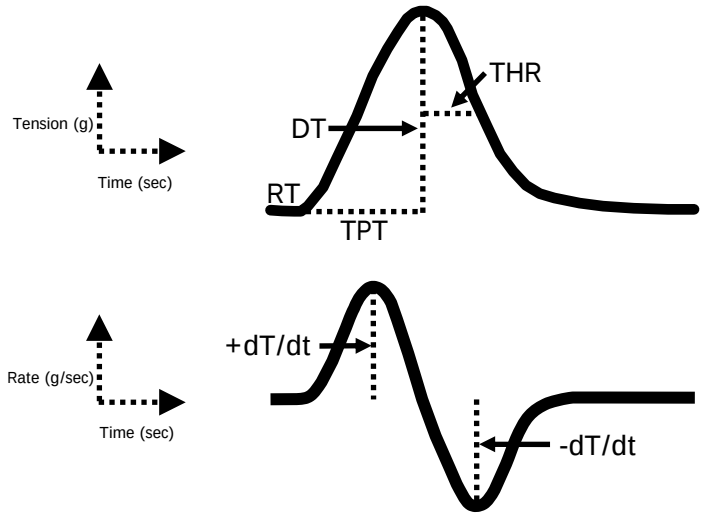


Figure 15. Individual muscle contraction showing six contractile parameters.

Muscle function was evaluated with respect to six contractile parameters. Resting tension (RT) is the tension produced by the muscle at rest. Developed tension (DT) is the force produced by the muscle during contraction. Time to peak tension (TPT) is the time it takes for the muscle to reach the peak of its contraction. Time to half relaxation (THR) is the time it takes for the muscle to get from that point of peak contraction to halfway through its relaxation. Peak rate of tension rise (+dT/dt) is the maximal rate of contraction (represented by the steepest point of the line leading to the peak of the muscle contraction). Peak rate of tension fall (-dT/dt) is the maximal rate of relaxation and is represented by the steepest point of the relaxation line moving back toward RT.

chloride ($MgCl_2$), 250mM sucrose, 20µg/mL leupeptin, 20µg/mL phenylmethylsulfonyl fluoride (PMSF), 20µg/mL bacitracin, 20µg/mL benzamidine) and homogenized using a Polytron homogenizer until there was little foam and no visible chunks of tissue floating in the homogenate. In order to minimize heat production and thereby the potential destruction of proteins, homogenization was carried out on ice in 3-second bursts with 5 seconds of rest in between activity to allow the homogenization probe to cool.

Homogenates were centrifuged at 4°C and 300 x g for 5 minutes so that heavier organelles such as nuclei and mitochondria precipitated out. Avoiding the pellet and layer of fat, supernatants were recovered and incubated in 0.5M KCl at 4°C for 15 minutes to remove myofilaments. These suspensions were centrifuged at 4°C and 40,000 x g for 15 minutes in order to pellet the membrane fraction. Supernatants were discarded, and pellets were added to chilled homogenization buffer B (composition: 20mM HEPES, 5mM EGTA, 12.5mM $MgCl_2$, 100mM sodium chloride (NaCl), 10µg/mL leupeptin, 20µg/mL PMSF, 20µg/mL bacitracin, 20µg/mL benzamidine).

Pellets were dounce homogenized in buffer B on ice, 10 times with a loose-fitting pestle and 10 times with a tight-fitting pestle, in order to purify the membrane fraction. Preparations were centrifuged at 4°C and 40,000 x g for 15 minutes, and pellets were recovered and dounce homogenized a second time (in buffer B, on ice, 10 times with a loose-fitting pestle and 10 times with a tight-fitting pestle) before a final centrifugation to refine the membrane preparation (again at 4°C and 40,000 x g for 15 minutes). Pellets were resuspended in buffer B + 10% glycerol (preserves the membrane preparations throughout storage) and completely solubilized using a motorized dounce homogenizer. Aliquots were stored at -80°C.

2.6 Measurement of Total Protein for Receptor Density Analysis

Total protein concentration was determined by the Lowry method using Bio-Rad reagents. Standards containing known concentrations (0, 500, 1000, 2000, 3000 and 4000 μg/mL) of bovine serum albumin (BSA) were pipetted into a 96-well microtiter plate in triplicate. Membrane preparations were diluted 1:2 in deionized water (dH_2O) and also added to the microtiter plate in triplicate. Reagent A, an alkaline copper tartrate solution, was added to each standard and sample to bind to the peptide bonds in all proteins. A dilute folin reagent, Reagent B was then added to each standard and sample to recognize Reagent A and to produce a characteristic blue color directly proportional to the amount of protein in the sample. After a 15-minute incubation at room temperature, absorbance was read at 750nm. Using the absorbance values for the known concentrations of BSA to generate a standard curve, the total protein concentration for each unknown sample was determined by interpolation.

2.7 Total Beta-Adrenergic Receptor Density

Beta-adrenergic receptor density was measured by radioligand binding analysis. In order to optimize conditions for radioligand binding, membrane titer assays were run. Specifically, six polypropylene tubes were arranged on ice for each membrane fraction to be measured. The assays were run in triplicate with three tubes used to measure total binding and three tubes to measure non-specific binding. A reaction buffer (HEM) (composition in mM: 20 4-(2-hydroxyethyl)-1-piperaineethanesulfonic acid (HEPES), 15 EGTA, 1.25 $MgCl_2$) plus 0.1% BSA was added to all six tubes for each sample. Propranolol (10^{-5}M), an unlabeled non-selective beta-adrenergic receptor blocker, was

then added to each non-specific binding tube. Next, a non-selective radiolabeled antagonist, ^{125}I-Cyanopindolol (ICYP) (30pM), was added to all reaction tubes. Finally, each membrane fraction was prepared in HEM + BSA buffer to a final protein concentration of 25μg/μL and added to all reaction tubes. All tubes were mixed and incubated in a shaking water bath at 37°C and 65 RPM for one hour. Following incubation, contents of each reaction tube were trapped onto glass fiber filter paper using a cell harvester. Forceps were used to carve out an individual filter for each reaction tube, and each filter was placed into a new polypropylene tube. The new polypropylene tubes were taken to a gamma radiation counter where the amount of radioactivity trapped on each piece of filter paper was determined. Each tube was read for one minute, and ^{125}I was measured in counts per minute. Triplicate values were averaged, and average non-specific binding was subtracted from average total binding to obtain a specific binding value for each sample. Because our laboratory has previously determined that the optimal running condition for radioligand binding assays is at 10% of the dissociation constant (K_d)[92], the amount of membrane necessary to run under this condition was calculated for each sample.

Next the radioligand binding assay was performed using seven doses of ICYP in the presence of 10^{-5}M propranolol. Similar to the membrane titer assay, everything was run in triplicate, with three tubes for measuring total binding and three tubes for measuring non-specific binding for each dose of ICYP for each sample (42 tubes / sample). The experiment was run with all tubes on ice. The highest concentration of ICYP (250pM) was prepared in HEM + BSA buffer, and subsequent doses were achieved via serial dilution (in pM: 125, 63, 31, 16, 8, 4). HEM + BSA buffer and propranolol were added

as described in the membrane titer assay. Each ICYP dose was added to six tubes (three total binding and three non-specific binding) for each sample, from the lowest concentration to the highest. Membrane preparations were prepared in HEM + BSA buffer according to the calculations from the membrane titer assay (at 10% K_d) and added to all 42 tubes for each sample. All tubes were mixed, incubated, harvested, and radioactivity was quantified again using the same procedure as in the membrane titer assay. In order to determine total density and K_d for each sample, Scatchard analyses were performed. **Figure 16** shows a typical saturation curve and Lineweaver-Burk plot generated in the Scatchard analysis.

2.8 Total Muscarinic Receptor Density

Much like the measurement of beta-adrenergic receptor density, total muscarinic receptor density was measured by radioligand binding assay, preceded by membrane titer analysis in order to optimize conditions. The membrane titer assay protocol was the same as that outlined in section 2.8, except that 1μM atropine was used as the unlabeled non-selective muscarinic receptor blocker, and 250pM ^{3}H-Quinuclidinyl benzilate (QNB) served as the non-selective radiolabeled antagonist. Also, following the cell harvesting step, individually carved filters were placed into scintillation vials and incubated in a hybridization oven at 42°C until dry (~5 minutes). All scintillation vials were then filled with Cytoscint scintillation fluid, mixed and wiped with a dryer sheet to prevent artificially high radiation readings from static. Before vials were read, they sat overnight on the benchtop at room temperature. The next day, all vials were taken to the scintillation counter where each vial was read for 2 minutes. Once again ^{3}H was

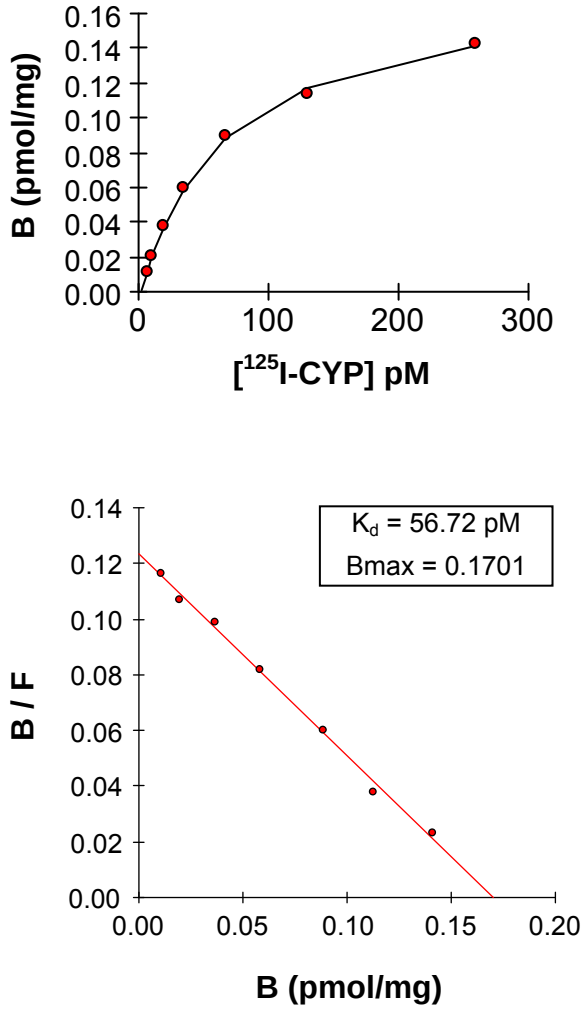


Figure 16. Typical saturation curve and Lineweaver-Burke plot.

Scatchard analyses generate a saturation curve and a Lineweaver-Burk plot. The saturation curve (top) illustrates the relationship between the concentration of radioactivity used and the amount of binding. The Lineweaver-Burk plot (bottom) illustrates the relationship between the amount of specific binding and the ratio of bound to free radioactivity. The x-intercept represents the density (Bmax) of the measured receptor, and the slope represents the binding affinity (K_d).

(Abbreviations: B = specific binding, B / F = the ratio of bound to free radioligand)

measured in counts per minute, and specific binding values were obtained by subtracting average non-specific binding from average total binding. This protocol was recently worked out in our laboratory, and it was determined that the optimal running condition for radioligand binding assays aimed at measuring muscarinic receptors is at 10% of the K_d (unpublished). The amount of membrane necessary to run under this condition was calculated for each sample.

To measure total muscarinic receptor density via radioligand binding, the same general process described in section 2.8 was employed. In this case, eight doses of ^{3}H-QNB were used in the presence of 1μM atropine. The highest dose of QNB was 1500pM, and non-logarithmic serial dilutions were used to prepare the remaining seven doses (in pM: 900, 540, 270, 135, 68, 27, 11). The cell harvesting steps were the same as those described above, and again, Scatchard analyses were performed to obtain final total binding and K_d values for each sample.

2.9 Tissue Homogenization for Western Blotting

Homogenization was done on ice in a Tris-EDTA (ethylenediaminetetraacetic acid) radioimmunoprecipitation assay (RIPA) buffer (composition: 20mM Tris, pH 7.4; 100mM NaCl; 5mM EDTA, pH 8.0; 10% glycerol; 50mM sodium fluoride (NaF); 1% Triton X-100; 0.1% sodium dodecyl sulfate (SDS); 1% sodium deoxycholate (DOC); 1mM sodium pyrophosphate ($Na_4P_2O_7$); 1mM sodium orthovanadate (Na_3VO_4); 10μg/mL PMSF) which also contained a standard cocktail of protease inhibitors for mammalian tissues (Sigma). All protease / phosphatase inhibitors were added just before use. Homogenization of human heart tissue samples (~ 300mg each) was performed on

ice using a Polytron homogenizer 3 times for 3 seconds each with 5-second rests between each homogenization. Homogenates were centrifuged at 4°C and 2,000 RPM for 10 minutes. Supernatants were recovered, and aliquots were stored at -80°C.

2.10 Measurement of Total Protein for Western Blotting

Total protein concentration was determined by the Lowry method using the Bio-Rad *DC* (detergent-compatible) protein assay kit. The process was the same as the protein assay used prior to receptor density measurements, except for the following: standard concentrations were 0, 200, 400, 600, 800 and 1,000 µg/mL BSA; homogenates were diluted 1:50; and Reagent S, a surfactant solution was added to Reagent A before it was added to the microtiter plate.

2.11 Western Blotting to Measure Calcium-Cycling Proteins

Homogenates were diluted in Laemmli buffer containing β-2-mercaptoethanol to a concentration of 4µg/µL. Proteins were separated on 8.0% (SERCA) or 7.0% (CALQ) SDS-PAGE running gels with 4% stacking gels. In the case of RYR, a 4-20% precast linear gradient gel (Bio-Rad) was used for protein separation. Gels were run at 125V in chilled running buffer (composition in mM: 19 tris(hydroxymethyl)aminomethane (Tris), 3.5 SDS, 192 glycine) until the dye front reached the bottom of gel (~90 minutes). Because RYR is such a large, globular protein, it was run at 125V for 2.5 hours on ice to avoid overheating.

Proteins were electroblotted onto polyvinylidene fluoride (PVDF) membranes in ice-cold transfer buffer (composition in mM: 17 Tris, 181glycine, 20% methanol (MeOH))

on ice at 100V for 2 hours (3 hours for RYR gels). Blots were blocked with 5% milk in tris buffered saline (TBS)-Tween for 2 hours and incubated in primary antibody at 4°C overnight on a rotisserie. The next day, blots were washed with TBS-Tween 4 times for 5 minutes each and incubated (shaking) in secondary antibody at room temperature for 1 hour. Exact antibody conditions can be found in **Table II**.

Blots were washed again with TBS-Tween 4 times for 5 minutes each. Blots were then developed via chemifluorescence using the Odyssey infrared scanner (Li-Cor, NE) and quantified with Odyssey version 3.0 software. Each sample was normalized to a non-failing sample that was run on every gel, which allowed for comparisons across gels.

2.12 Data Analysis

Biofeedback, Quality of Life and Clinical Data

For all before and after biofeedback training analyses, paired t-tests were performed. If the data were not normally distributed, then a Wilcoxon matched-pairs signed rank test was used to make comparisons.

Across-session analyses were performed using a one-way repeated measures analysis of variance (ANOVA) with Newman-Keuls post-hoc testing when necessary. Freidman tests with Dunn's Multiple Comparison post-hoc tests were performed when the data did not follow a normal distribution.

Inpatient versus outpatient comparisons were made using a 2-way repeated measures ANOVA and Bonferroni post-hoc analyses.

In all cases, alpha was set at 0.05, and analyses were performed using GraphPad Prism software, version 5.02 (San Diego, CA).

Table II. Western Blot Antibody Conditions

Host	Primary Antibody	1° Ab Dilution	2° Ab Dilution
Mouse	Anti-SERCA	1:30K	1:20K
Rabbit	Anti-NCX	1:10K	1:10K
Mouse	Anti-RYR	1:3K	1:10K
Rabbit	Anti-CALQ	1:10K	1:20K

SERCA, RYR and CALQ primary antibodies were from Thermo Scientific (Golden, CO). NCX primary antibody was from Swant (Switzerland). All secondary antibodies were from Li-Cor Biosciences (Lincoln, NE).

Removing Artifact from Heart Rate Variability Data

Before comparisons were made with respect to heart rate variability, the raw data had to be analyzed for potential artifacts. Artifacts were defined as inter-beat intervals that did not match up with a pulse on the blood volume pulse trace. Raw inter-beat interval data were scrolled through, and the cursor on the blood volume pulse trace "jumped" from pulse to pulse. When the cursor did not land on a pulse, then this was considered to be an artifact, and it was manually removed using one of three strategies: (1) if the IBI value was longer than those nearby, then it was split into two equal IBI values, (2) if the IBI value was longer than those nearby and the consecutive IBI was shorter than those nearby (or vice versa; if the two consecutive IBIs were a relatively short value followed by a relatively long value), then the two consecutive IBIs were averaged, and (3) if there were two consecutive short IBI values, then the IBIs were added. All heart rate variability artifact removal was performed using CardioPro Infiniti: HRV Analysis Module for BioGraph Infiniti software (Thought Technology, Montreal, QC).

Biological Data

A one-way ANOVA was used to compare non-failing, failing, failing + LVAD and failing + BF groups with respect to muscle function, total receptor density and K_d (beta-adrenergic and muscarinic), as well as the relative presence of calcium-cycling proteins. When necessary, Newman-Keuls post-hoc tests were performed. If any one of the groups did not follow a normal distribution, as determined by the Kolmogorov-Smirnov test, then a Kruskal-Wallis test was performed with Dunn's Multiple Comparison post-hoc

analysis. Alpha was set at 0.05, and analyses were performed using GraphPad Prism software, version 5.02 (San Diego, CA).

Correlation Data

To determine whether success with biofeedback was related to biological change, linear regressions were performed. The correlations were analyzed using GraphPad Prism software, version 5.02 (San Diego, CA), and alpha was again set at 0.05.

CHAPTER III

RESULTS

3.1 Biofeedback Data

Table III shows demographic data on the 35 patients enrolled in the biofeedback portion of this study. Highlighted in gray are the 20 patients who completed the entire biofeedback-assisted stress management training protocol (all eight sessions of biofeedback), and only these 20 patients were used in the biofeedback data analysis. Summary demographics on these 20 patients can be found in **Table IV**.

Respiration Rate

The first physiologic modality focused on during biofeedback-assisted stress management training was respiration. **Figure 17** shows that patients were able to lower their average respiration rate from 14.9 ± 2.9 breaths per minute in session 1 (before biofeedback training) to 9.4 ± 2.7 breaths per minute in session 8 (after biofeedback training) ($p < 0.001$). In order to determine how many training sessions it took before a significant change occurred, average respiration rate was also evaluated across sessions.

Table III. Demographics of the 35 End-Stage Heart Failure Patients Enrolled in the Study

	Age	Sex	Race	Diagnosis	LVEF (%)	Medications
IP 1	59	M	W	ICM	15	ACE I, BB
IP 2	66	M	W	ICM	10	AAR, BB, DIG
IP 3	61	M	W	ICM	20	ACE I, BB, DIG
IP 4	53	F	B	DCM	15	ACE I, BB
IP 5	61	F	W	DCM	10	ACE I, BB, DIG
IP 6	58	M	W	DCM	30	ACE I, BB
IP 7	27	M	W	CONG	10	N/A
IP 8	46	M	W	Restrictive CM	60	N/A
IP 9	52	F	W	DCM	15	AAR, ACE I, BB, DIG
IP 10	45	M	W	CONG	55	BB, DIG
IP 11	65	M	W	DCM	15	ARB, BB, DIG
IP 12	63	F	W	ICM	20	ACE I, BB, DIG
IP 13	28	F	B	DCM	20	INO
IP 14	69	M	W	ICM	15	AAR, ACE I, BB, DIG, INO
IP 15	61	M	B	DCM	10	BB, INO
IP 16	68	M	W	ICM	51	AAR, BB, DIG, INO
IP 17	50	M	B	DCM	20	AAR, BB, DIG
IP 18	61	M	W	Amyloidosis	20	N/A
IP 19	55	F	W	HCM	10	AAR, BB
IP 20	66	M	W	DCM	30	ACE I, BB
IP 21	58	M	W	DCM	37	AAR, BB
OP 1	59	M	W	DCM	20	ACE I, BB, DIG
OP 2	20	M	H	DCM	18	ACE I, BB, DIG
OP 3	60	F	W	DCM	25	Not Listed for Transplant
OP 4	52	M	W	DCM	15	ACE I, BB
OP 5	67	F	B	DCM	10	AAR, ACE I, BB, DIG, OVD
OP 6	43	M	B	ICM	12	ACE I, BB
OP 7	44	M	W	CONG	15	ACE I, BB, DIG
OP 8	61	M	W	ICM	20	ACE I, DIG
OP 9	49	M	W	ICM	20	BB
OP 10	20	M	W	Restrictive CM	30	ACE I, BB
OP 11	46	F	B	DCM	10	BB
OP 12	64	M	W	DCM	30	ACE I, BB, DIG
OP 13	66	M	W	DCM	10	AAR, ACE I, BB
OP 14	61	M	W	ICM	19	BB, OVD

IP = inpatient; OP = outpatient; M = male; F = female; W = white; B = black; H = Hispanic; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; HCM = hypertrophic cardiomyopathy; CONG = congenital; AAR = anti-arrhythmic; ACE I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; BB = beta blocker; DIG = digoxin; INO = inotrope; OVD = other vasodilator

= patients who completed all 8 sessions of biofeedback

Table IV. Demographics of the 20 End-Stage Heart Failure Patients Who Completed All 8 Sessions of Biofeedback

Patient Status (n = 20)	
Inpatient	12 (60%)
Outpatient	8 (40%)
Age, y	56 ± 10
Gender	
Male	17 (85%)
Female	3 (15%)
Race	
White	16 (80%)
Black	4 (20%)
NYHA	
III	17 (85%)
IV	3 (15%)
Diagnosis	
DCM	9 (45%)
ICM	6 (30%)
CONG	3 (15%)
Restrictive CM	1 (5%)
Amyloidosis	1 (5%)
LVEF, %	23 ± 15
Medications	
AAR	4 (20%)
ACE I	9 (45%)
ARB	1 (5%)
BB	15 (75%)
DIG	10 (50%)
INO	3 (15%)

DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; CONG = congenital; AAR = anti-arrhythmic; ACE I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; BB = beta blocker; DIG = digoxin; INO = inotrope

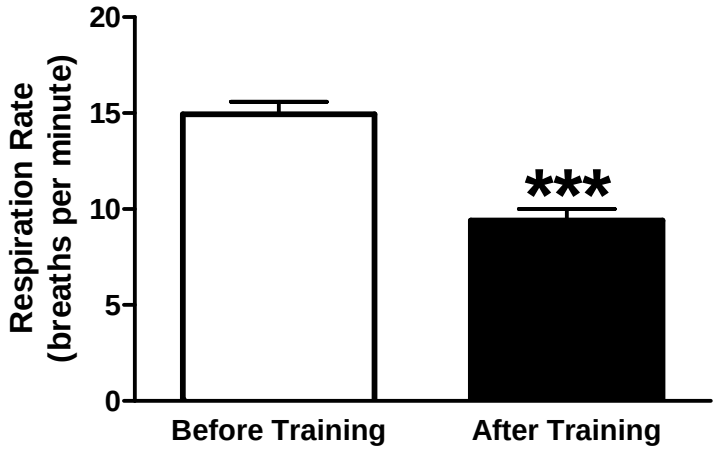


Figure 17. Average respiration rate during self-relaxation before and after biofeedback training.

Patients lowered their respiration rate from 14.9 ± 2.9 breaths per minute before biofeedback training to 9.4 ± 2.7 breaths per minute after biofeedback training ($p < 0.001$).

After only three sessions of biofeedback training (session 4), patients were breathing at a significantly lower rate than they were in session 1 before they had any formal training ($p < 0.05$), and respiratory rate continued to decrease throughout the remaining sessions (**Figure 18**).

Respiration rate was also evaluated as the percentage of time patients spent meeting specific criteria before and after biofeedback training. Self-relaxation portions of the psychophysiologic assessment, including the 5-minute self-relaxation at the beginning of the session and the rest periods (each five minutes in length) following each mental stress task, were aggregated and labeled as "relaxation time." As shown in **Figure 19**, patients spent a significantly greater percentage of relaxation time breathing at rates at or less than 10 ($p < 0.001$), 8 ($p < 0.001$) and 6 ($p < 0.01$) breaths per minute after biofeedback training.

Clinical significance was also analyzed by assigning a letter grade to each patient's average breathing rate during the 5-minute self-relaxation of the psychophysiologic assessment before and after biofeedback training. **Table V** illustrates that 80% of patients made clinical improvements in their respiration rate from session one to session eight.

Inpatients vs. Outpatients

Respiration rate data were also analyzed by patient status (inpatients vs. outpatients). As shown in **Figure 20**, there were no significant differences between these groups ($p = 0.86$), but both inpatients and outpatients significantly lowered their respiration rate following biofeedback training. Inpatients went from 15.3 ± 3.5 breaths

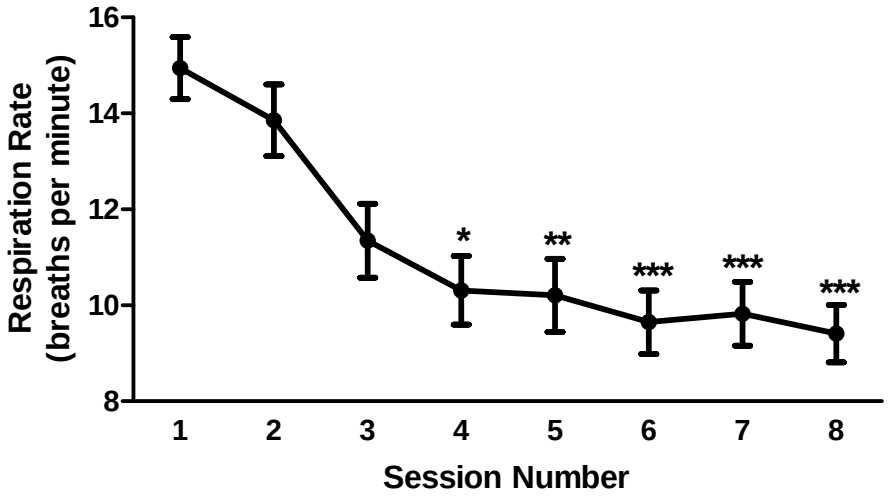


Figure 18. Average respiration rate during self-relaxation across all biofeedback sessions.

Average respiration rate among patients decreased across sessions, reaching a significantly lower rate (relative to session 1) in session 4 ($p < 0.05$) and continued to decrease in session 5 ($p < 0.01$) and throughout the remaining three sessions ($p < 0.001$).

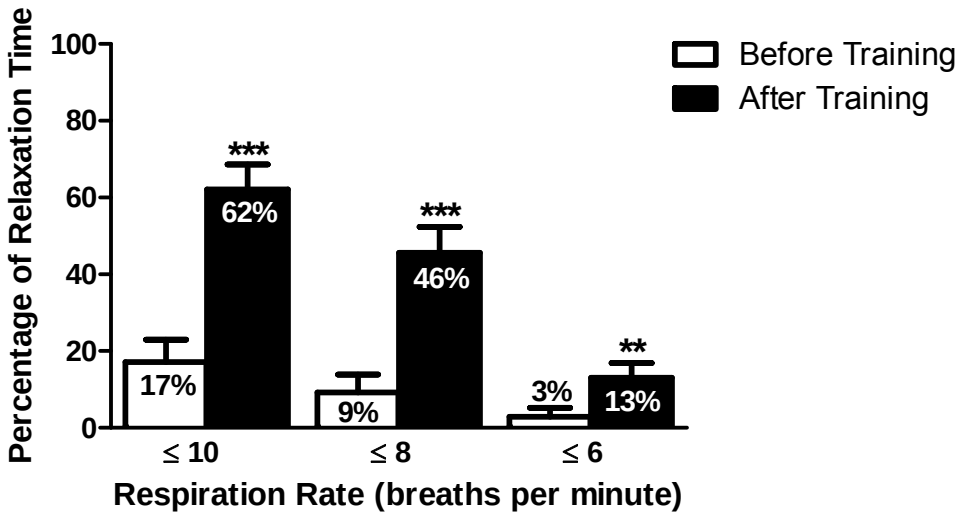


Figure 19. Percentage of relaxation time patients spent breathing at lower rates before and after biofeedback training.

Patients spent significantly more time breathing at or less than 10, 8 and 6 breaths per minute, following biofeedback training (** $p < 0.01$ and *** $p < 0.001$ vs. Before Training).

Table V. Clinical Analysis of Respiration Rate Before and After Biofeedback Training

Change in Clinical Rank	*Number (%) of Patients*
D → A	6 (30)
D → B	4 (20)
C → A	2 (10)
C → B	2 (10)
B → A	2 (10)
No Change	4 (20)

KEY: A = 9 or lower; B = 10-12; C = 13-15; D = 16 or greater breaths per minute

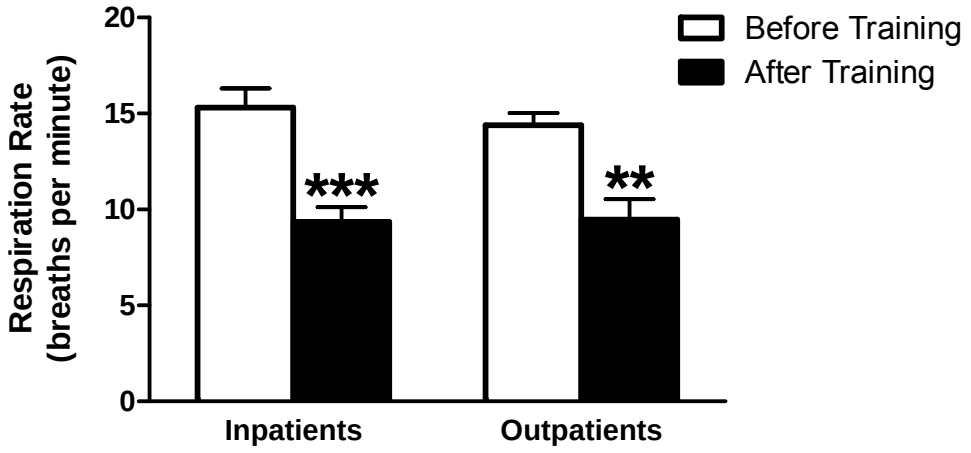


Figure 20. Average respiration rate during self-relaxation before and after biofeedback training in inpatients and outpatients.

No significant differences between inpatients and outpatients were found, but both groups significantly lowered their respiration rate following biofeedback training (inpatients: $p < 0.001$ vs. Before Training; outpatients: $p < 0.01$ vs. Before Training).

per minute in session 1 to 9.4 ± 2.6 breaths per minute in session 8 ($p < 0.001$), and outpatients went from 14.4 ± 1.8 breaths per minute in session 1 to 9.5 ± 3.0 breaths per minute in session 8 ($p < 0.01$).

In the across-sessions analysis, inpatients were able to significantly lower their respiration rate earlier in biofeedback training than outpatients. As shown in **Figure 21**, inpatient breathing rate reached statistical significance by session 4 ($p < 0.05$), and outpatients were breathing at a significantly lower rate in session 6 ($p < 0.05$).

No differences between inpatients and outpatients were found in the percentage of relaxation time patients met specific criteria (percent counter) analysis. In all cases, both inpatients and outpatients spent significantly more relaxation time breathing at lower rates after biofeedback training (p-values vs. before training are shown in **Table VI**).

Digital Peripheral Temperature

In addition to respiration rate, digital peripheral temperature was also analyzed before and after biofeedback training as well as across all biofeedback sessions. **Figure 22** shows that there were no significant changes in finger temperature following biofeedback training ($p = 0.72$) or across biofeedback sessions (overall p-value = 0.99). Two patients were able to make a clinically significant improvement in finger temperature, moving from a range of 80-90°F in session 1 to ≥ 90°F in session 8.

Inpatients vs. Outpatients

No significant differences in temperature were found between inpatients and outpatients following biofeedback training ($p = 0.34$) or across biofeedback sessions (IP:

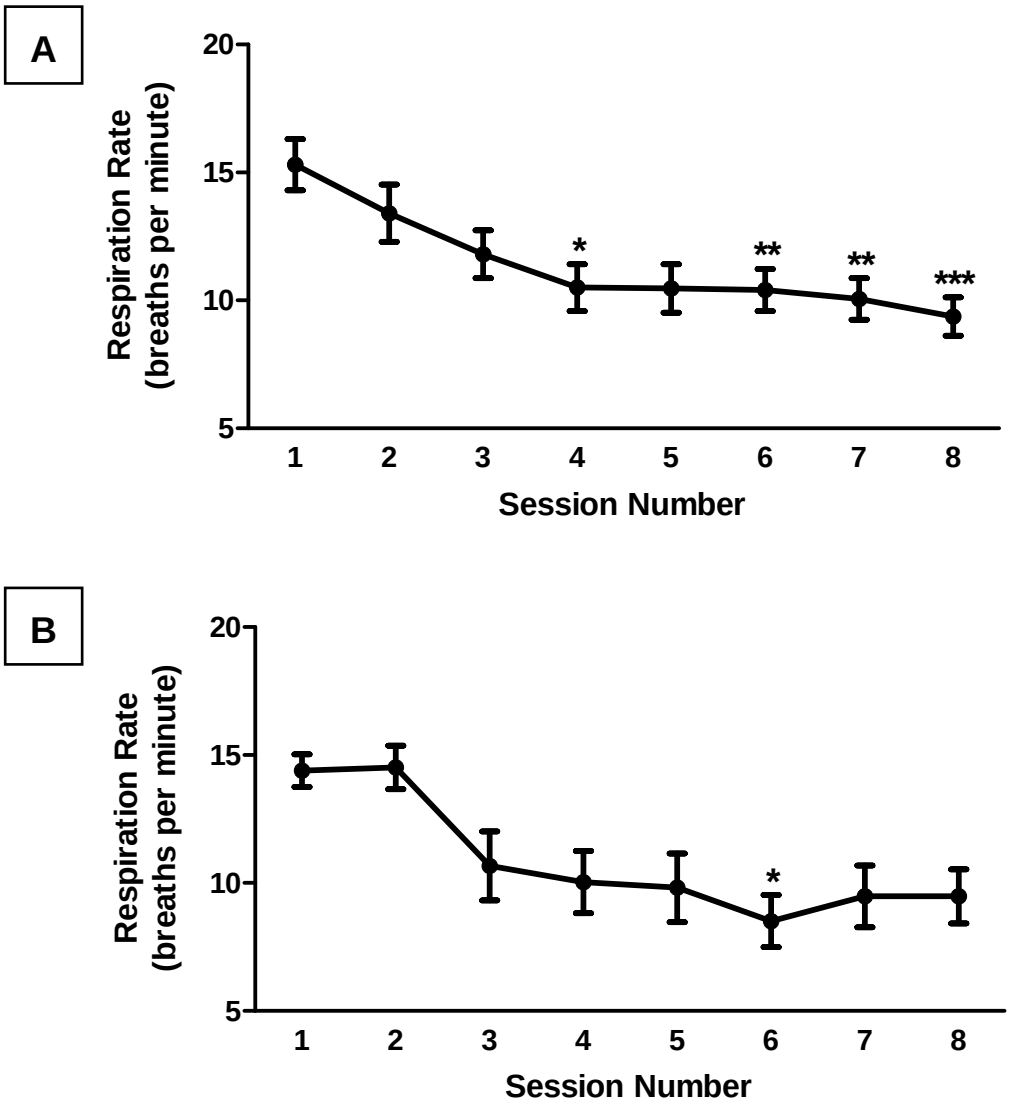


Figure 21. Average respiration rate during self-relaxation across all biofeedback sessions in inpatients and outpatients.

With respect to session 1, inpatients (A) were able to lower respiration significantly in session 4 ($p < 0.05$), and outpatients (B) significantly lowered respiration rate in session 6 ($p < 0.05$).

Table VI. Respiration Rate Percent Counter Summary for Inpatients and Outpatients

≤ 10 breaths per minute		≤ 8 breaths per minute		≤ 6 breaths per minute	
IPs	OPs	IPs	OPs	IPs	OPs
$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.05$

The p-values shown are vs. before training.

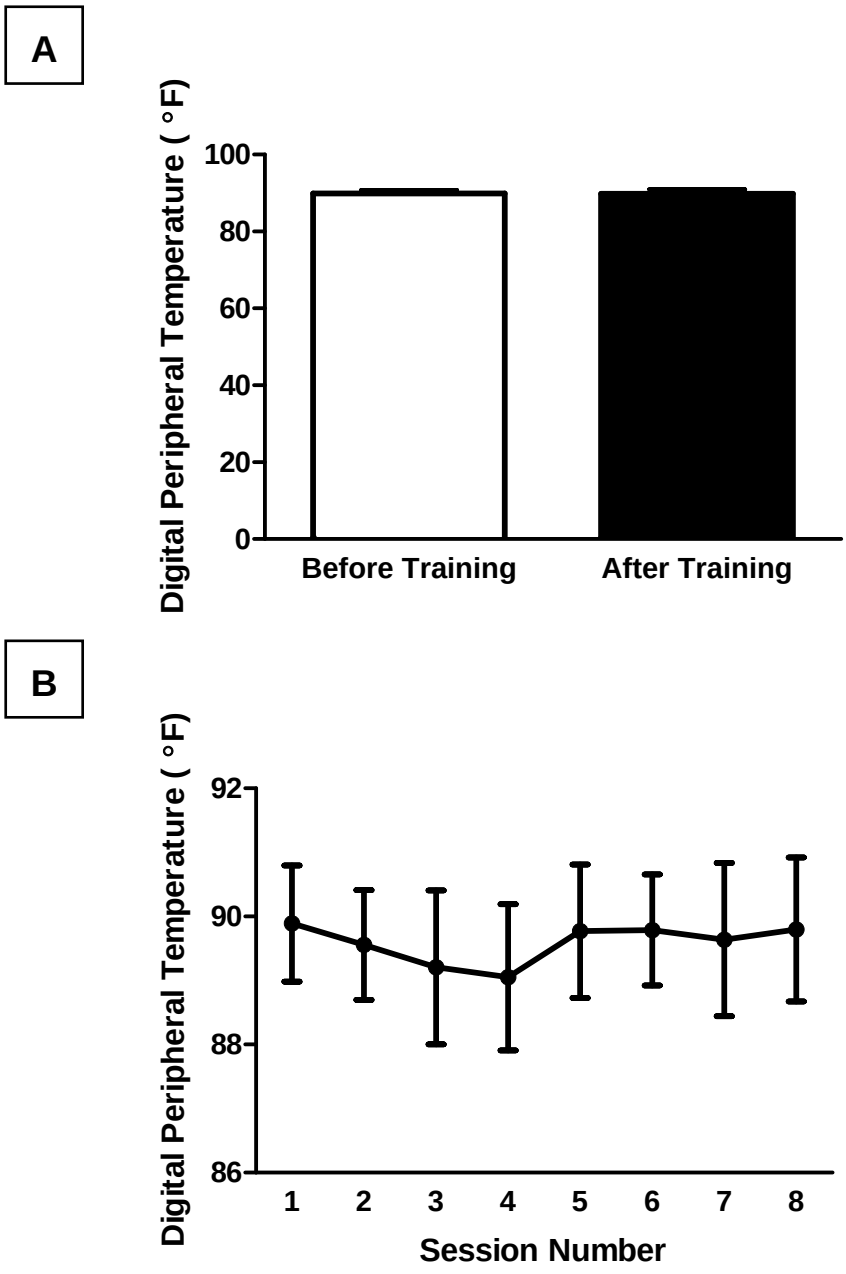


Figure 22. Average finger temperature following and throughout biofeedback training.

Digital peripheral temperature did not change significantly (A) following biofeedback training ($p = 0.72$) or (B) across biofeedback sessions ($p = 0.99$).

p = 0.63; OP: p = 0.94). Of the two patients who made clinical improvements in their finger temperature, one was an inpatient, and one was an outpatient.

Skin Conductance

Due to safety precautions, skin conductance sensors were not used in patients who had a pacemaker. As a result, skin conductance was only able to be measured in two patients (both inpatients), and therefore it was analyzed across biofeedback sessions only. No significant changes were made in skin conductance throughout biofeedback sessions ($p = 0.39$) as shown in **Figure 23**, but the average skin conductance value at each session was below 2μS, a value that represents relaxed physiology.

Heart Rate Variability

The standard deviation of the inter-beat interval (SDNN) during the 5-minute self-relaxation of each session was calculated and compared before and after biofeedback training as well as across all biofeedback sessions. **Figure 24** shows that average SDNN increased significantly after biofeedback training ($p < 0.05$), from 32 ± 22 milliseconds to 44 ± 23 milliseconds. On an individual basis, 5 out of 20 patients (25%) increased their SDNN from an unhealthy range (0-50 msec) to a moderately healthy range (50-100 msec), as defined by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology.[110]

When analyzing SDNN across all biofeedback sessions, 5 patients were dropped from the analysis because there was missing data in one of their eight sessions, and therefore these 5 patients could not be included in a repeated measures analysis of

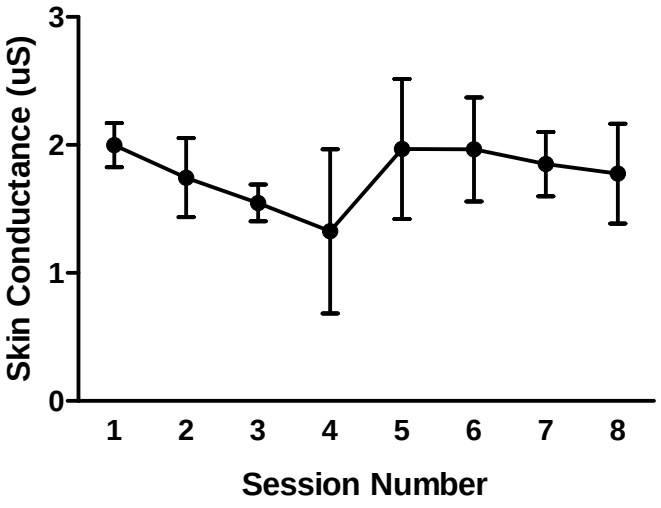


Figure 23. Average skin conductance during self-relaxation across all biofeedback sessions.

Skin conductance (n = 2) did not change significantly across biofeedback sessions (p = 0.39).

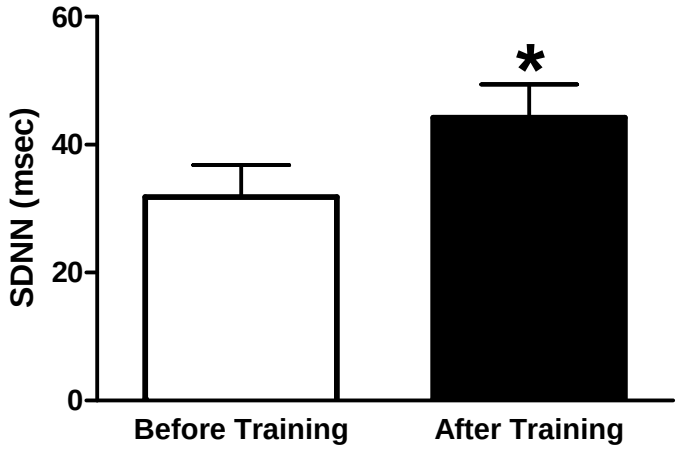


Figure 24. Average SDNN during self-relaxation before and after biofeedback training.

On average, patients' SDNN increased from 32 ± 22 msec to 44 ± 23 msec following biofeedback training ($p < 0.05$ vs. Before Training).

variance. The across-sessions analysis of the 15 remaining patients for which all data were present showed that SDNN increased significantly across sessions (overall p-value = 0.03), as shown in **Figure 25**.

SDNN data were also analyzed across sessions using all 20 patients (1) by replacing missing values with the same value as the previous session and (2) by replacing missing values with an average of the values from the session before and after the missing session, but neither of these analyses showed a significant change over time ((1) p = 0.09; (2) p = 0.37).

Inpatients vs. Outpatients

When the SDNN data (n = 15) was separated by patient status, no significant differences were found before and after biofeedback training (p = 0.69). Although not significant, outpatient SDNN before training (24 ± 18 msec) was lower than inpatient SDNN before training (37 ± 24 msec) (**Figure 26**). Across biofeedback sessions, outpatients showed a significant increase in SDNN (overall p-value = 0.01) that was not present in the inpatient sample (p = 0.62) (**Figure 27**).

Psychophysiologic Reactivity and Recovery

In order to assess the effects of biofeedback-assisted stress management training on patients' response to mental stress, instantaneous heart rate was analyzed before, during and after each mental stress task in the psychophysiologic assessment before and after biofeedback training. **Figure 28** shows that there was no significant difference in baseline heart rate prior to the start of either psychophysiologic assessment (p = 0.45).

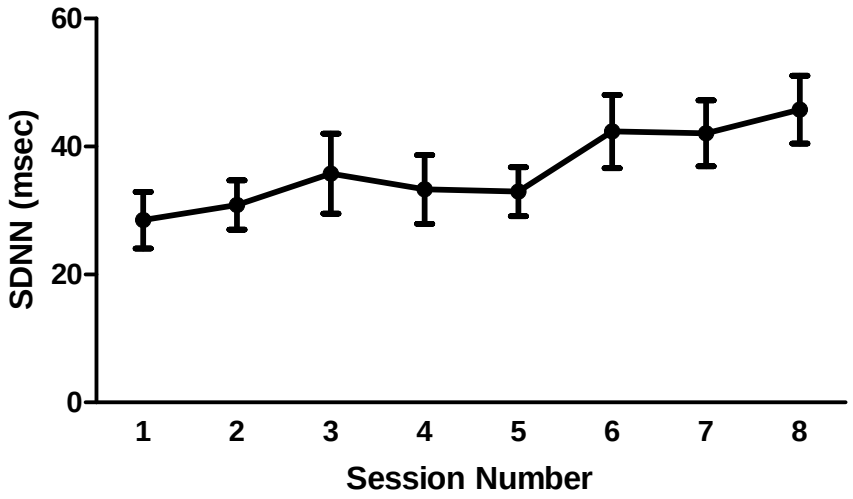


Figure 25. Average SDNN during self-relaxation across all biofeedback sessions.

SDNN (n = 15) increased significantly across sessions (overall p-value = 0.03).

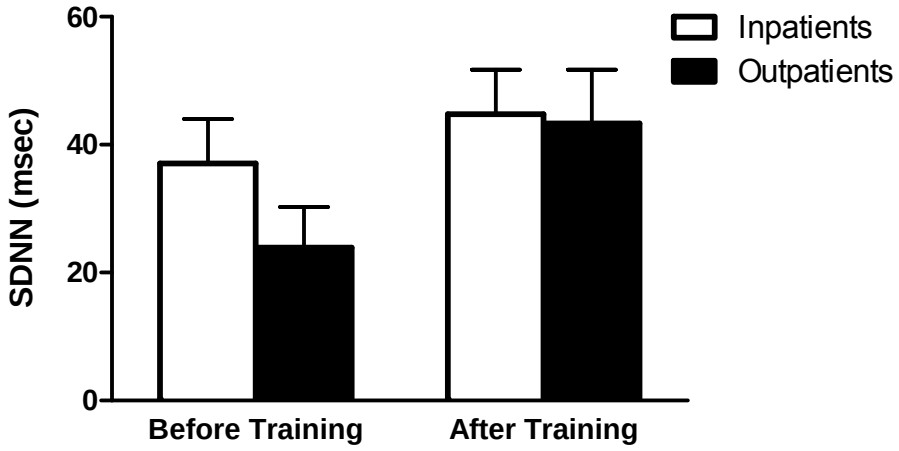


Figure 26. Average SDNN during self-relaxation before and after biofeedback training in inpatients and outpatients.

Outpatient (n = 7) SDNN before training, while not significant (p = 0.32), is lower than inpatient (n = 8) SDNN before training.

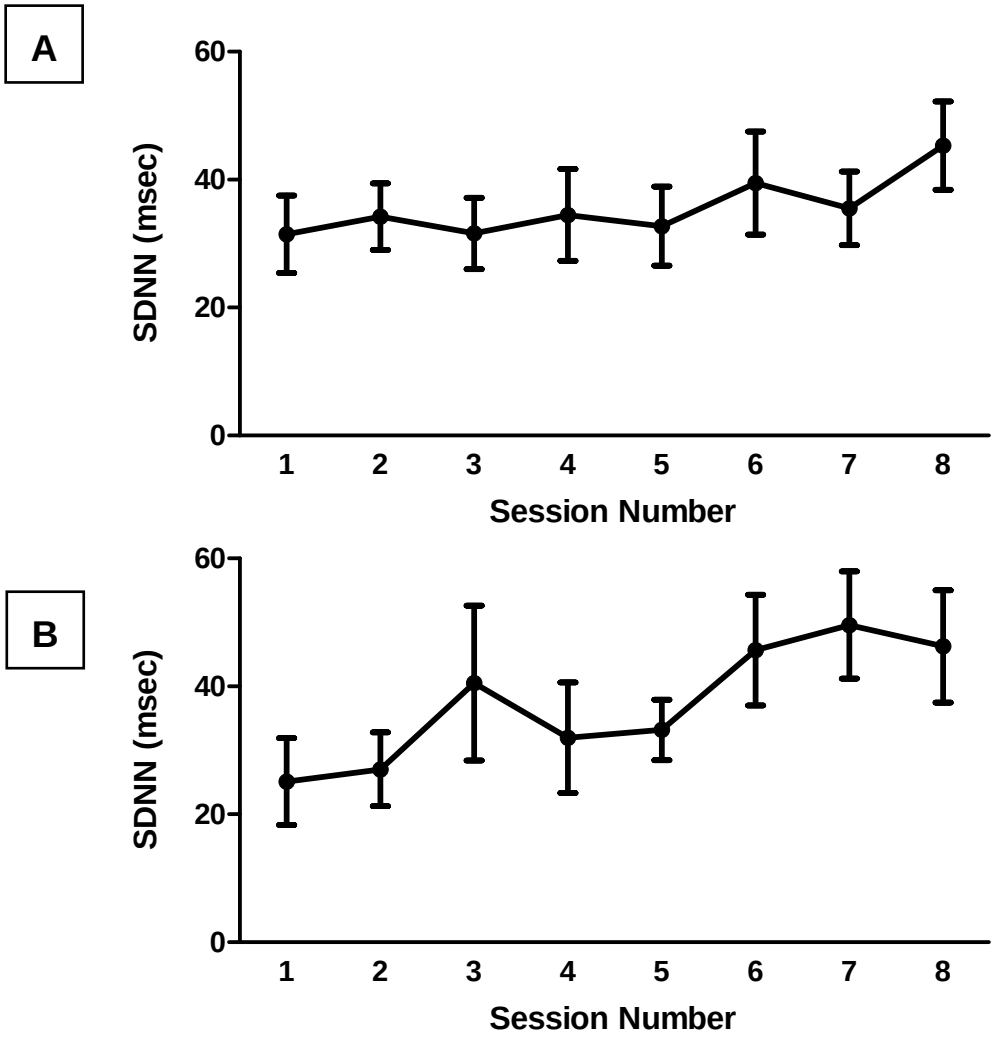


Figure 27. Average SDNN during self-relaxation across all biofeedback sessions in inpatients and outpatients.

(A) Inpatients (n = 8) did not change SDNN across biofeedback sessions (p = 0.62). (B) Outpatients (n = 7) significantly increased SDNN across biofeedback sessions (p = 0.01).

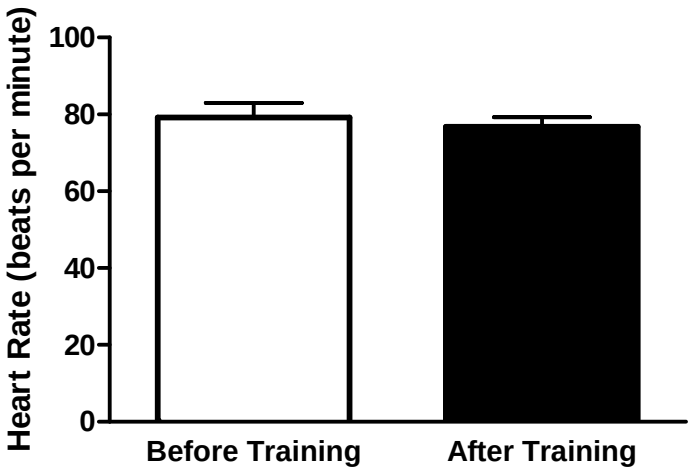


Figure 28. Average heart rate during five-minute baseline in psychophysiologic assessment before and after biofeedback training.

Baseline heart rate was not significantly different in the psychophysiologic assessment before and after biofeedback training ($p = 0.45$).

Cardiovascular reactivity to each stressor was measured by subtracting the average heart rate before the stressor from the average heart rate during the stressor, and both absolute change and percent change were analyzed. Following biofeedback training, cardiovascular reactivity (absolute change) decreased in response to the Stroop Color Word Test (5.5 ± 8.5 to 3.8 ± 7.3 beats per minute) and increased in response to the Serial Sevens (2.5 ± 8.4 to 3.5 ± 7.6 beats per minute) and Stressful Event Recall (4.9 ± 8.8 to 8.4 ± 6.8 beats per minute) tasks, however none of these changes reached statistical significance ($p = 0.34$, 0.87, and 0.16, respectively).

Using this same method of calculating absolute change in cardiovascular reactivity, trends were established for each stressor, and the results are summarized in Table **VII**. Overall, not counting the patients whose reactivity did not change, about half the remaining patients reacted less to mental stress following biofeedback training, and about half the patients reacted more.

The most reliable measure of cardiovascular reactivity is an aggregate percent change score calculated by adding the percent change response for all three stressors and comparing this value before and after biofeedback training.[70] **Figure 29** shows that overall, cardiovascular reactivity did not change after biofeedback training ($p = 0.86$).

Recovery from mental stress was also evaluated by subtracting the average heart rate before each stressor from the average heart rate after the stressor. Absolute and percent change scores were calculated, with positive values indicating that the baseline heart rate had not been reached (average heart rate after the stressor still higher than the average heart rate before the stressor), and negative scores indicating that the baseline heart rate had been surpassed (average heart rate after the stressor was even lower than

Table VII. Trends in Cardiovascular Reactivity

	Reacted Less After Training	No Change in Reactivity	Reacted More After Training
Stroop	9	4	7
Math	6	4	10
Recall	9	2	9
	24 / 60 (40%)	**10 / 60 (17%)**	**26 / 60 (43%)**

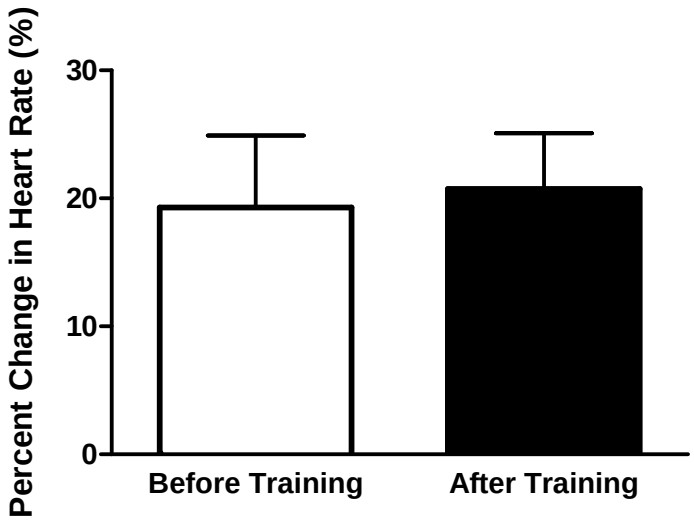


Figure 29. Overall cardiovascular reactivity to mental stress following biofeedback training.

There was no significant change in cardiovascular reactivity to mental stress following biofeedback training ($p = 0.86$). Data are expressed as an aggregate percent change.

the average heart rate before the stressor).

Following biofeedback training, patients recovered less from the Stroop Color Word Test (0.23 ± 3.7 to 1.8 ± 5.0 beats per minute) and more from the Serial Sevens (-0.41 ± 4.3 to -1.8 ± 6.3 beats per minute) and Emotional Event Recall (-5.6 ± 7.1 to -8.1 ± 7.3 beats per minute) stressors, however none of these changes were significant ($p = 0.39$, 0.78, and 0.30, respectively). The percent change aggregate recovery data (calculated by adding the percent change response for all recoveries), shown in **Figure 30**, also indicates that there was no significant change in cardiovascular recovery from mental stress following biofeedback training ($p = 0.28$).

Inpatients vs. Outpatients

When the data were separated into inpatients and outpatients, no significant differences were found in average baseline heart rate prior to mental stress before or after biofeedback training ($p = 0.97$).

Figure 31 shows the aggregate percent change scores for cardiovascular reactivity and recovery separated by patient status. Although not significant ($p = 0.24$), inpatients reacted more to mental stress following biofeedback training, and outpatients reacted less. With respect to cardiovascular recovery, overall results were significant ($p = 0.01$); inpatients recovered from mental stress more than outpatients.

3.2 Homework Data

Table VIII shows the number of homework sheets that were turned in by each patient. The patients on the right in bold type are those who completed all eight sessions

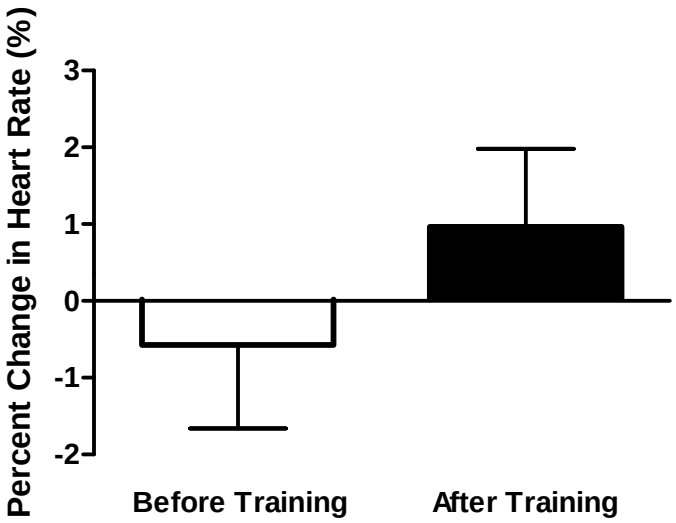


Figure 30. Overall cardiovascular recovery from mental stress following biofeedback training.

There was no significant change in cardiovascular recovery from mental stress following biofeedback training ($p = 0.28$). Data are expressed as an aggregate percent change.

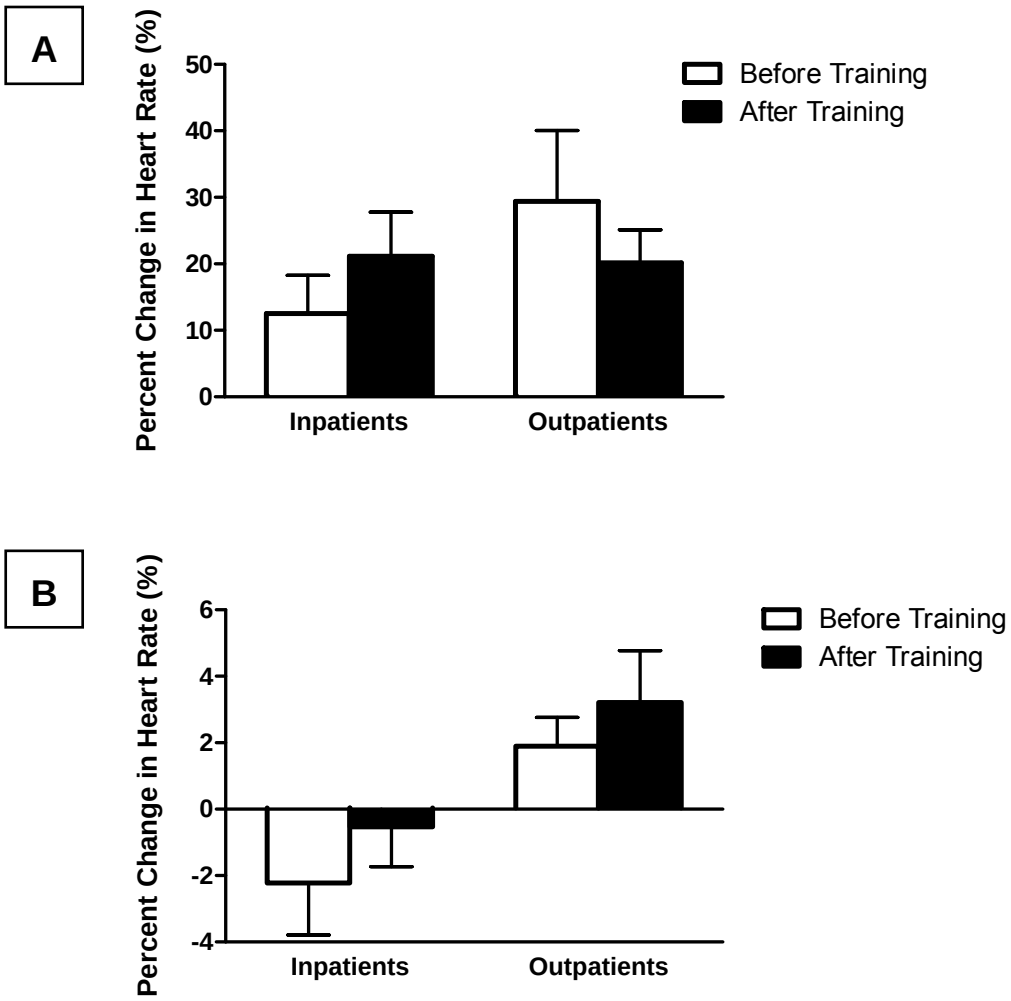


Figure 31. Overall cardiovascular reactivity and recovery before and after biofeedback training in inpatients and outpatients.

Cardiovascular reactivity to mental stress (A) increased in inpatients and decreased in outpatients following biofeedback training, although the differences were not statistically significant. Cardiovascular recovery from mental stress (B) was significantly greater in inpatients relative to outpatients ($p = 0.01$).

Table VIII. Number of Homework Sheets Turned In

IP 2	2	OP 4	-	**IP 1**	**5**	**IP 17**	**0**
IP 5	4	OP 5	2	**IP 3**	**23**	**IP 18**	**5**
IP 6	0	OP 6	-	**IP 4**	**3**	**OP 1**	**34**
IP 9	0	OP 10	0	**IP 7**	**0**	**OP 3**	**34**
IP 12	0	OP 14	0	**IP 8**	**12**	**OP 7**	**28**
IP 13	0			**IP 10**	**0**	**OP 8**	**17**
IP 19	0			**IP 11**	**0**	**OP 9**	**34**
IP 20	6			**IP 14**	**6**	**OP 11**	**16**
IP 21	9			**IP 15**	**0**	**OP 12**	**4**
OP 2	0			**IP 16**	**0**	**OP 13**	**12**

Patients highlighted in bold type are the 20 patients who completed all 8 sessions of biofeedback

of biofeedback.

The 20 patients who completed all eight sessions of biofeedback were put into quartiles based on the number of homework sheets they turned in, and as shown in **Figure 32**, outpatients did more homework than inpatients. This result reached statistical significance when the average number of homework sheets turned in was compared. Inpatients turned in 5 ± 7 sheets, and outpatients turned in 22 ± 12 sheets ($p < 0.01$).

3.3 Quality of Life Data

SF-36

Table IX lists the mean of each SF-36 aggregate score before and after biofeedback training and its corresponding p value. Note that aggregate scores are out of 100, and an increase in score reflects a greater level of functioning. No significant differences were found.

Inpatients vs. Outpatients

Each aggregate score was also separated by patient status, and eight out of ten scores did not show any significant differences. As shown in **Figures 33 and 34** respectively, inpatients had a lower social functioning score ($p < 0.05$) and a greater general health score ($p < 0.05$) when compared to outpatients.

The eight aggregate scores for which clinically important differences have been established[123] are shown in **Table X**. For both inpatients and outpatients, some changes in aggregate scores reflected clinical improvement and some reflected clinical regression.

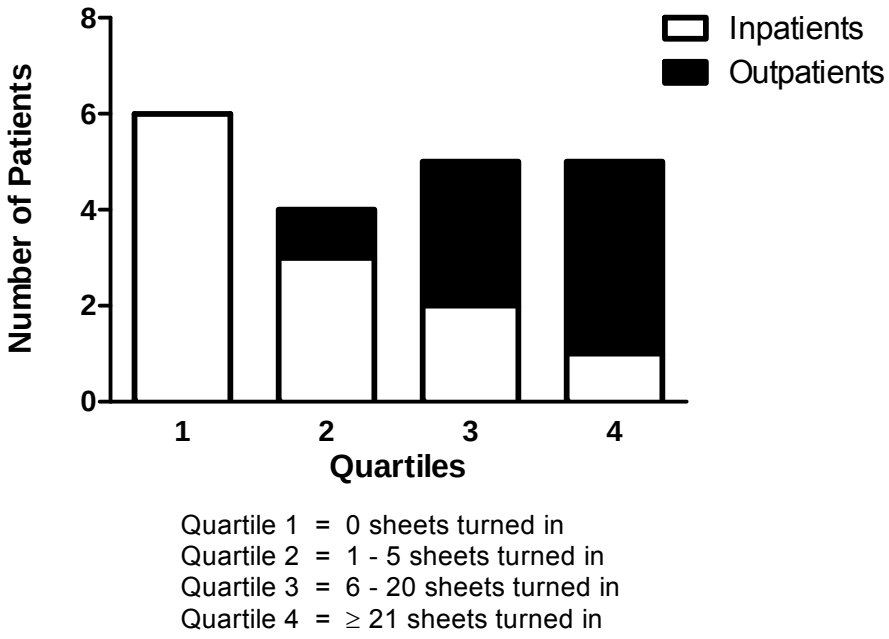


Figure 32. Quartile distribution of homework turned in by inpatients and outpatients.

Outpatients turned in more homework than inpatients. This difference was statistically significant when the average number of homework sheets turned in was compared. On average, inpatients turned in 5 ± 7 sheets, and outpatients turned in 22 ± 12 sheets ($p < 0.01$).

Table IX. Aggregate Scores on SF-36 Before and After Biofeedback Training

Aggregate Score	Before Biofeedback Training	After Biofeedback Training	p - value
Physical Functioning	29.5 ± 22.4	30.5 ± 22.35	0.72
Role Limitations (Physical)	10.5 ± 25.4	11.8 ± 21.0	0.71
Role Limitations (Emotional)	45.0 ± 43.6	38.3 ± 43.6	0.35
Energy / Fatigue	34.4 ± 19.8	40.1 ± 25.0	0.17
Emotional Well-Being	67.5 ± 20.0	68.8 ± 18.1	0.76
Social Functioning	48.2 ± 31.5	50.8 ± 30.7	0.20
Pain	58.5 ± 25.6	65.6 ± 24.0	0.22
General Health	34.4 ± 19.3	35.8 ± 21.4	0.45
PHYSICAL SUMMARY	28.2 ± 5.89	29.8 ± 7.59	0.23
MENTAL SUMMARY	46.1 ± 10.8	46.1 ± 10.8	0.97

Score data are presented as average ± standard deviation

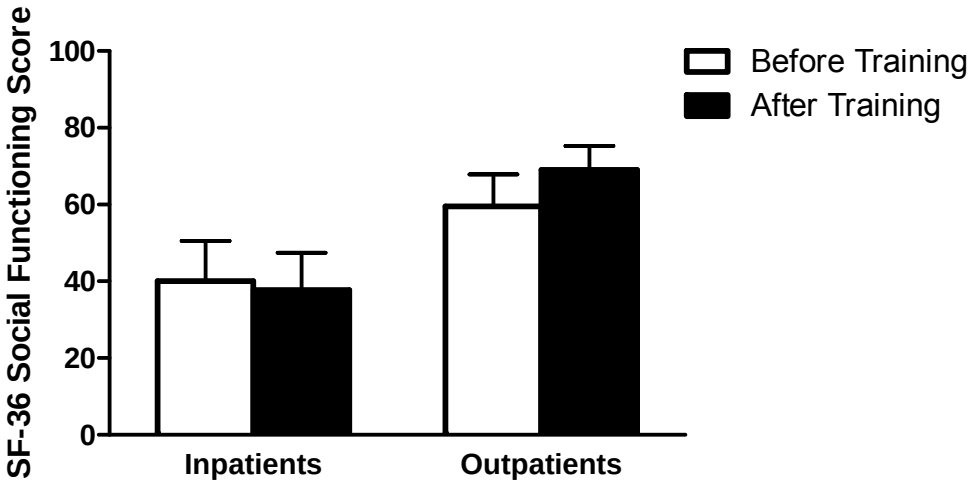


Figure 33. SF-36 social functioning aggregate score before and after biofeedback training in inpatients and outpatients.

Inpatients reported lower social functioning than outpatients ($p < 0.05$), and this did not change with biofeedback training.

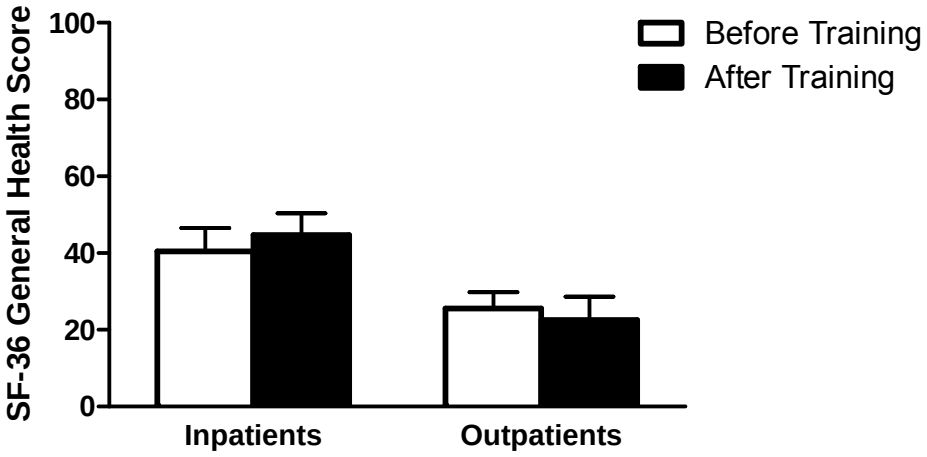


Figure 34. SF-36 general health aggregate score before and after biofeedback training in inpatients and outpatients.

Inpatients reported greater general health relative to outpatients ($p < 0.05$), and this did not change with biofeedback training.

Table X. Clinical Analysis of SF-36 Aggregate Scores

SF-36 Summary Score	PF	RLP	RLE	EF	EWB	SF	P	GH
Minimal Clinical Improvement	1 IP	1 IP 3 OP		1 IP	1 IP 1 OP	2 IP 2 OP	3 IP 2 OP	3 IP 2 OP
Moderate Clinical Improvement			1 IP	1 IP	1 IP 1 OP		2 IP	1 IP
Large Clinical Improvement	1 IP	2 IP						
Minimal Clinical Decline	1 OP	1 OP		1 IP 1 OP	3 IP 1 OP		1 IP 3 OP	1 IP 1 OP
Moderate Clinical Decline	1 IP		1 IP		1 OP			1 IP 1 OP
Large Clinical Decline			1 IP 1 OP			1 IP		

PF = Physical Functioning, RLP = Role Limitations (Physical), RLE = Role Limitations (Emotional), EF = Energy / Fatigue, EWB = Emotional Well-Being, SF = Social Functioning, P = Pain, GH = General Health, IP = Inpatient, OP = Outpatient

Kansas City Cardiomyopathy Questionnaire

Table XI lists the mean of each Kansas City Cardiomyopathy (KCCM) aggregate score before and after biofeedback training and its corresponding p-value. Once again the aggregate scores are out of 100, and an increase in score reflects a greater level of functioning. Note that no significant differences were found.

Inpatients vs. Outpatients

KCCM aggregate scores were also separated by patient status, and eight out of ten summary scores did not show any significant differences. **Figures 35 and 36** show that inpatients had a lower quality of life score ($p < 0.01$) and a higher clinical summary score ($p < 0.05$) relative to outpatients.

Clinically significant differences in each of the ten KCCM summary scores are outlined in **Table XII** and separated by patient status. Clinical improvement and clinical decline were seen among aggregates for both inpatients and outpatients.

3.4 Subjective Data

Throughout both psychophysiologic assessments, patients were asked to report their level of relaxation after each activity (self-relaxation, stressors and subsequent rests) using a 5-point Likert scale with 1 being not at all relaxed and 5 being completely relaxed. As shown in **Figure 37**, patients felt significantly more relaxed after biofeedback training for every psychophysiologic assessment activity except for the self-relaxation ($p = 0.15$) and the stressful event recall ($p = 0.13$).

Psychophysiologic assessment activities were also grouped into stressors and

Table XI. Aggregate Scores on KCCM Before and After Biofeedback Training

Aggregate Score	Before Biofeedback Training	After Biofeedback Training	p - value
Physical Limitation	42.8 ± 22.5	46.7 ± 22.4	0.25
Symptom Stability	56.5 ± 16.3	55.2 ± 19.6	0.82
Symptom Frequency	61.2 ± 22.8	64.3 ± 24.1	0.41
Symptom Burden	66.6 ± 19.5	65.8 ± 22.6	0.84
Total Symptom	64.0 ± 20.2	65.0 ± 22.1	0.77
Self-Efficacy	85.7 ± 17.7	92.0 ± 11.5	0.16
Quality of Life	40.4 ± 19.7	40.3 ± 22.6	0.97
Social Limitation	32.7 ± 28.4	28.1 ± 19.9	0.60
OVERALL SUMMARY	47.4 ± 14.1	47.5 ± 13.3	0.96
CLINICAL SUMMARY	57.1 ± 16.2	59.4 ± 16.9	0.49

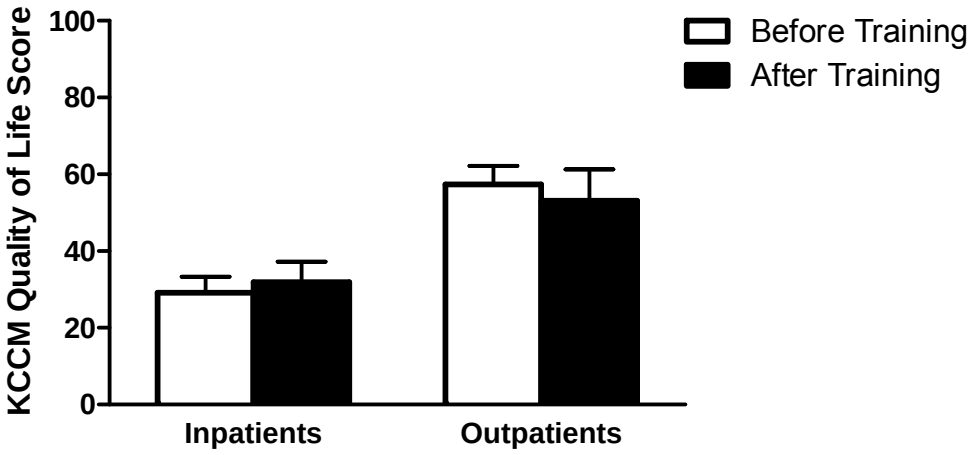


Figure 35. KCCM quality of life aggregate score before and after biofeedback training in inpatients and outpatients.

Overall, inpatients reported a lesser quality of life than outpatients ($p < 0.01$), and this did not change with biofeedback training.

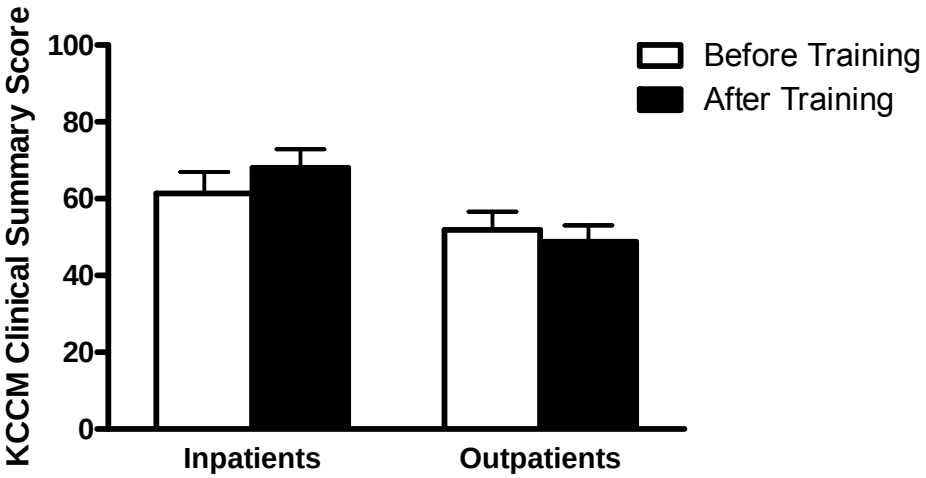


Figure 36. KCCM clinical summary aggregate score before and after biofeedback training in inpatients and outpatients.

Inpatient clinical summary score was higher than outpatient clinical summary score ($p < 0.05$), and this did not change with biofeedback training.

Table XII. Clinical Analysis of KCCM Aggregate Scores

KCCM Summary Score	PL	SS	SF	SB	TSS	SE	QOL	SL	OSS	CSS
Clinical Improvement	5 IP 4 OP	1 IP 2 OP	6 IP 3 OP	6 IP 3 OP	6 IP 3 OP	4 IP 3 OP	6 IP 3 OP	3 IP 1 OP	6 IP 0 OP	6 IP 4 OP
No Change	6 IP 1 OP	7 IP 4 OP	3 IP 3 OP	3 IP 1 OP	4 IP 2 OP	6 IP 4 OP	3 IP 1 OP	4 IP 3 OP	0 IP 6 OP	3 IP 0 OP
Clinical Decline	1 IP 3 OP	3 IP 2 OP	3 IP 2 OP	3 IP 4 OP	2 IP 3 OP	2 IP 1 OP	3 IP 4 OP	3 IP 4 OP	4 IP 2 OP	1 IP 4 OP

PL = Physical Limitation, SS = Symptom Stability, SF = Symptom Frequency, SB = Symptom Burden, TSS = Total Symptom Score, SE = Self-Efficacy, QOL = Quality of Life, SL = Social Limitation, OSS = Overall Summary Score, CSS = Clinical Summary Score, IP = Inpatient, OP = Outpatient

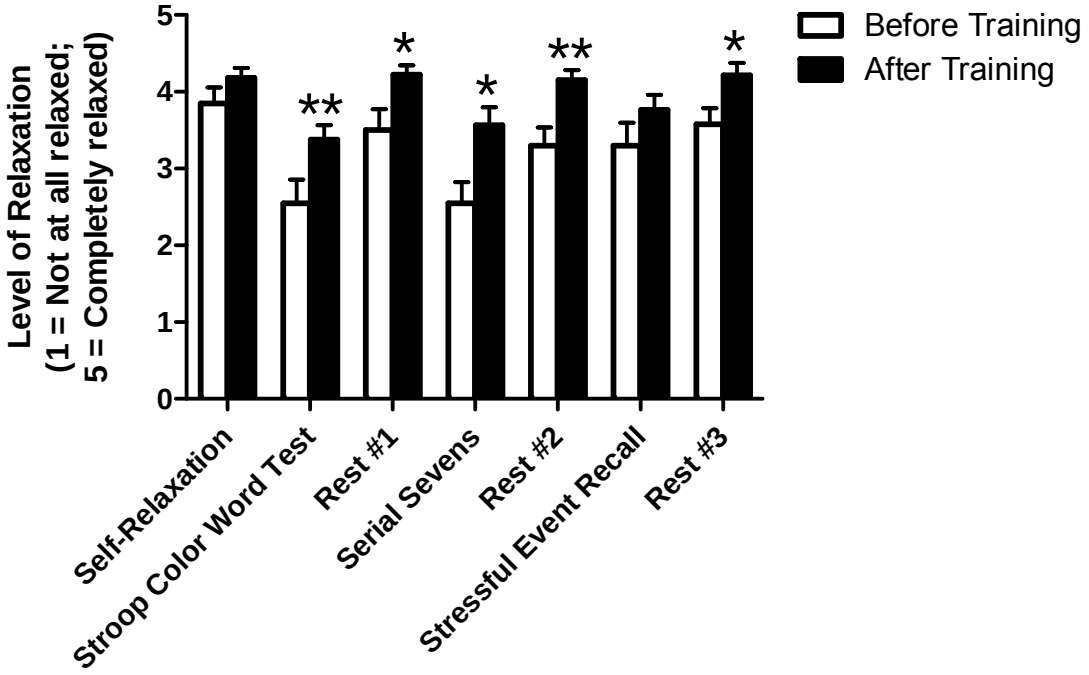


Figure 37. Self-reported relaxation level throughout psycho-physiologic assessment before and after biofeedback training.

Patients reported being more relaxed throughout the psychophysiologic assessment following biofeedback training ($*p < 0.05$ and $**p < 0.01$).

relaxing activities. This analysis showed that patients reported being more relaxed after relaxing activities than stressful activities ($p < 0.01$), independent of biofeedback training. Grouped activities were then analyzed before and after biofeedback training, and **Figure 38** shows that patients were significantly more relaxed after biofeedback training for both stressful activities ($p < 0.001$) and relaxing activities ($p < 0.01$).

The same relaxation Likert scale was used to gauge patients' relaxation levels at the beginning and the end of each training session. **Figure 39** shows that patients were more relaxed at the end of training sessions than they were at the beginning.

A similar 5-point Likert scale was used to evaluate patients' mood at the beginning and end of each training session. On this Likert mood scale, 1 represented sad, and 5 represented happy. **Figure 40** shows that patients reported being in a better mood at the end of biofeedback training sessions.

Inpatients vs. Outpatients

Both inpatients and outpatients reported feeling more relaxed after biofeedback training for each psychophysiologic assessment activity. The difference reached statistical significance for inpatients with respect to the Serial Sevens ($p < 0.05$), Rest #2 ($p < 0.01$) and Rest #3 ($p < 0.05$) activities. Significantly greater relaxation for the outpatients was reported following the Stroop Color Word Test ($p < 0.05$).

In the grouped analysis of psychophysiologic assessment activities, there was no difference between inpatient and outpatient responses to stressors ($p = 0.31$) or to relaxing activities ($p = 0.32$). In both cases, a significant training effect was shown. Both inpatients and outpatients reported being more relaxed after biofeedback training with

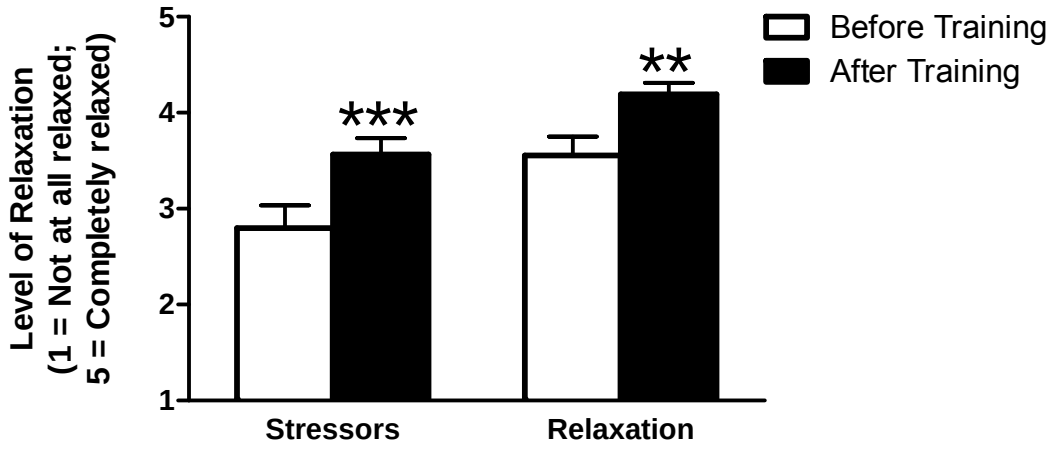


Figure 38. Self-reported relaxation level for stressful and relaxing psychophysiologic assessment activities before and after biofeedback training.

Patients reported being more relaxed following biofeedback training for both stressful (*** $p < 0.001$) and relaxing (** $p < 0.01$) activities of the psychophysiologic assessment.

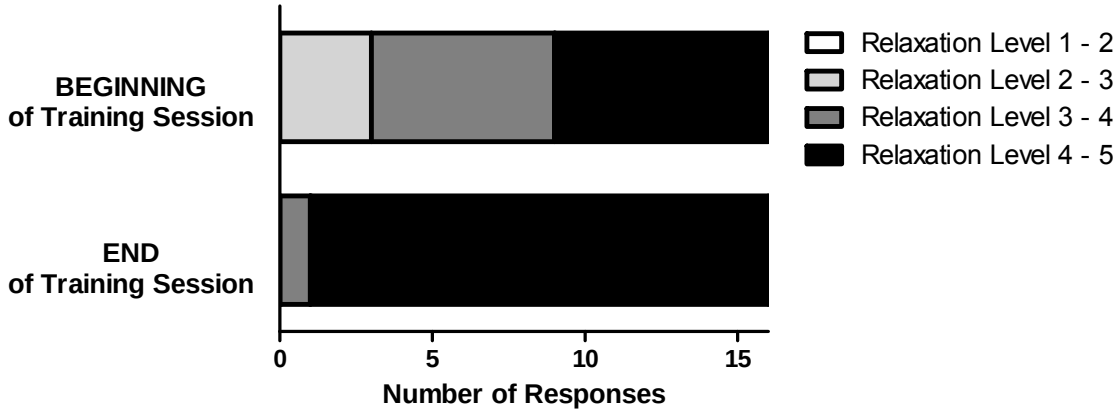


Figure 39. Average self-reported relaxation level throughout training sessions.

On average, patients reported being more relaxed at the end of biofeedback training sessions than at the beginning.

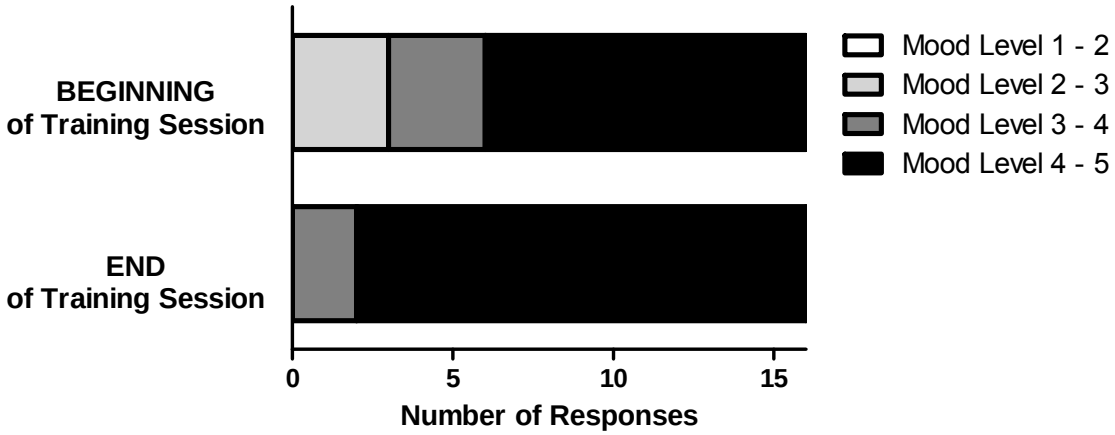


Figure 40. Average self-reported mood throughout training sessions.

On average, patients reported being in a better mood at the end of biofeedback training sessions than at the beginning.

respect to both stressors (overall p-value = 0.002) and relaxing activities (overall p-value = 0.003). These relationships were only significant for inpatients, however. Inpatients were significantly more relaxed after stressors and relaxing activities after biofeedback training (both stressor and relaxing activity p-values < 0.05).

3.5 Clinical Data

Clinical data were collected in outpatients only. As a reference for the remaining analyses, **Table XIII** provides a checklist for what data were collected on each patient in the study. Patients who completed all eight sessions of biofeedback are highlighted in gray.

Six Minute Walk Test

Patients (n = 7) walked an average of 910 ± 421 feet before biofeedback training and 961 ± 318 feet after biofeedback training (p = 0.81) as shown in **Figure 41**. For patients with heart failure, 130 feet is the clinical cut-off for improvement or regression in functional capacity,[3,91] and when analyzed as individuals using this criterion, **Figure 42** shows that three patients (highlighted in red, blue and green) made clinical improvements in their six-minute walk distance (red: 1080 to 1270 feet; blue: 420 to 690 feet; green: 200 to 500 feet).

Plasma Norepinephrine

Figure 43 shows that patients' average plasma norepinephrine level (n = 8) was 501 ± 312 pg/mL before biofeedback training and 470 ± 288 pg/mL after biofeedback

Table XIII. Data Collected on All Patients Enrolled in the Study

PT ID	SA #1	# T	SA #2	6MW	NE	SF-36	KCCM	Tx	M	R	W
IP 1	X	6	X			X	X	X	X	X	X
IP 2	X	4						X	X	X	X
IP 3	X	6	X			X	X	X	X	X	X
IP 4	X	6	X			X	X	X	X	X	X
IP 5	X	2						X			
IP 6	X	4						X	X	X	X
IP 7	X	6	X			X	X				
IP 8	X	6	X					X	X	X	X
IP 9	X	1									
IP 10	X	6	X			X	X				
IP 11	X	6	X				X	X	X		X
IP 12	X	1						X			
IP 13	X	4									
IP 14	X	6	X			X					
IP 15	X	6	X			X	X	X	X	X	X
IP 16	X	6	X			X	X				
IP 17	X	6	X			X	X				
IP 18	X	6	X			X	X				
IP 19	X	0						X			
IP 20	X	5						X	X	X	X
IP 21	X	4						X	X	X	X
OP 1	X	6	X		X	X	X				
OP 2	X	5						X	X	X	X
OP 3	X	6	X	X	X	X	X				
OP 4	*Consented, but never returned our call to schedule his first appointment…*										
OP 5	X	4									
OP 6	*Consented, but received a heart transplant before his first appointment…*										
OP 7	X	6	X	X	X	X	X				
OP 8	X	6	X	X	X	X	X				
OP 9	X	6	X	X	X	X	X				
OP 10	X	1									
OP 11	X	6	X	X	X	X	X				
OP 12	X	6	X	X	X	X	X				
OP 13	X	6	X	X	X	X	X				
OP 14	X	2						X			

IP = Inpatient; OP = Outpatient; SA = Stress Assessment (#1 - Before Biofeedback; #2 - After Biofeedback); #T = Number of Training Sessions Completed; 6MW = Six Minute Walk Test; NE = Plasma Norepinephrine; SF-36 = Short-Form 36; KCCM = Kansas City Cadiomyopathy Questionnaire; Tx = Heart Transplant; M = Muscle Function Experiments; R = Beta / Muscarinic Receptors; W = Western Blots

Note: Biological experiments were done if the patient had at least 4 out of 6 biofeedback training sessions before receiving a heart transplant.

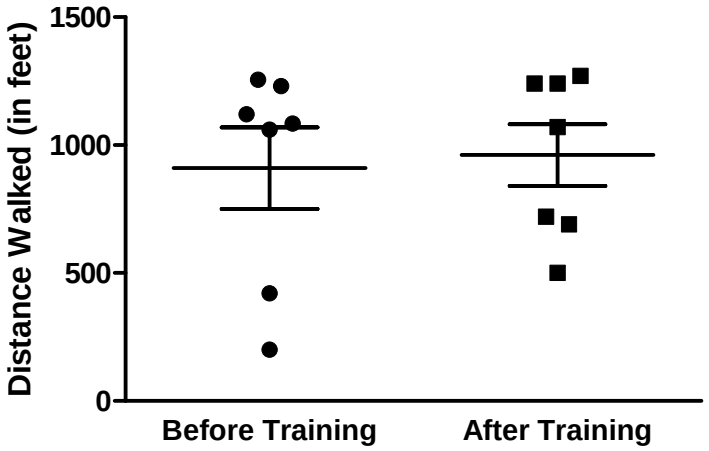


Figure 41. Average six minute walk distance before and after biofeedback training.

Average six minute walk distance did not change following biofeedback training (n = 7; p = 0.81).

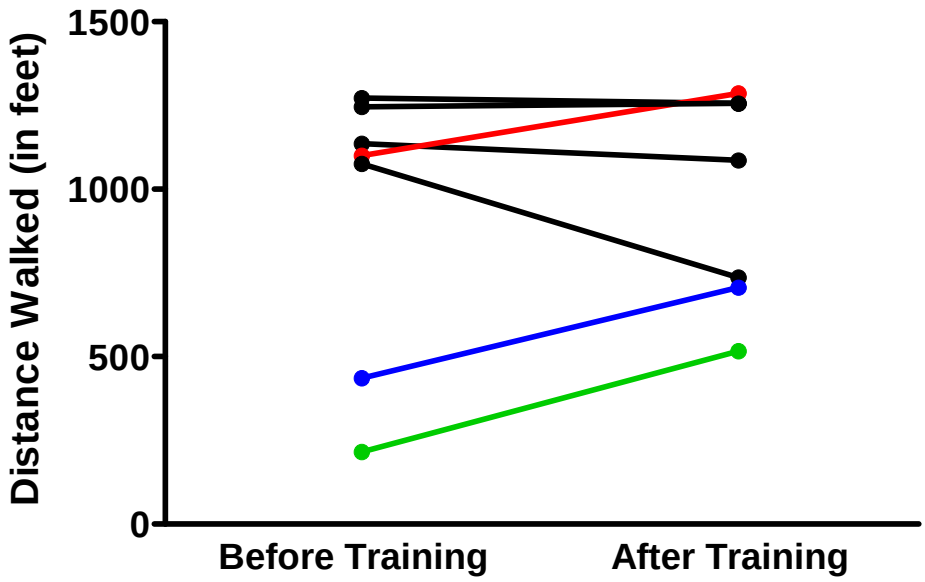


Figure 42. Individual six minute walk distance before and after biofeedback training.

Three patients (highlighted in red, blue and green) made clinical improvements in six minute walk distance following biofeedback training.

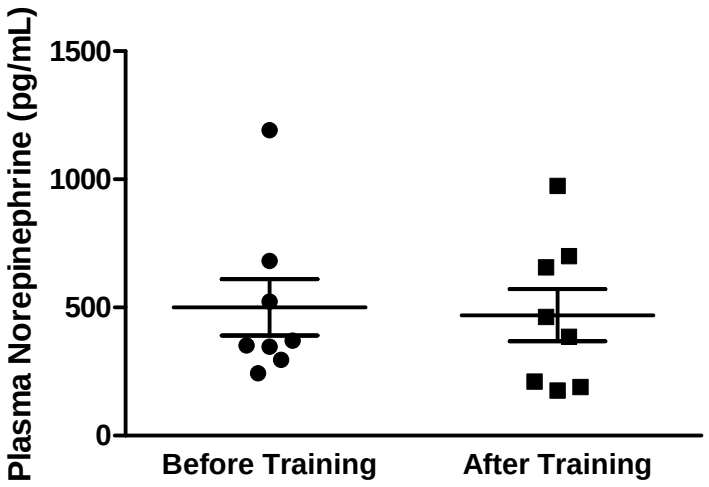


Figure 43. Average plasma norepinephrine level before and after biofeedback training.

Average norepinephrine level did not change following biofeedback training ($n = 8$; $p = 0.72$).

training (p = 0.72). Although not the same three patients who made clinical improvements in their six minute walk distance, another three patients' plasma norepinephrine levels decreased (improved) following biofeedback training as shown in **Figure 44** (red: 1192 to 701 pg/mL; blue: 352-175; green: 296-189).

3.6 Biological Data

Tables XIV and XV display detailed and summarized patient demographics, respectively, for the groups analyzed in the biological experiments. A total of 47 patients were studied, including 12 donors (non-failing – NF), 12 heart failure patients (failing), 12 heart failure patients who were hemodynamically supported with a left ventricular assist device prior to heart transplant (F + LVAD), and 11 heart failure patients who received biofeedback-assisted stress management training (F + biofeedback) prior to cardiac transplantation. Average age among groups ranged from 48 to 56 years old, with the majority of patients being white males with dilated cardiomyopathy. Average left ventricular ejection fraction for patients in the non-failing group was 61%, and for the three failing groups, average LVEF ranged from 12-19%.

Muscle Function

Muscle function experiments were conducted in order to test the response of freshly dissected trabecular muscles to sympathetic nervous system (beta-adrenergic) stimulation. This was accomplished by adding a synthetic analogue of norepinephrine, called isoproterenol (ISO), to the muscles and measuring various contractile parameters. In order to ensure that the contractile responses measured were due to our experimental

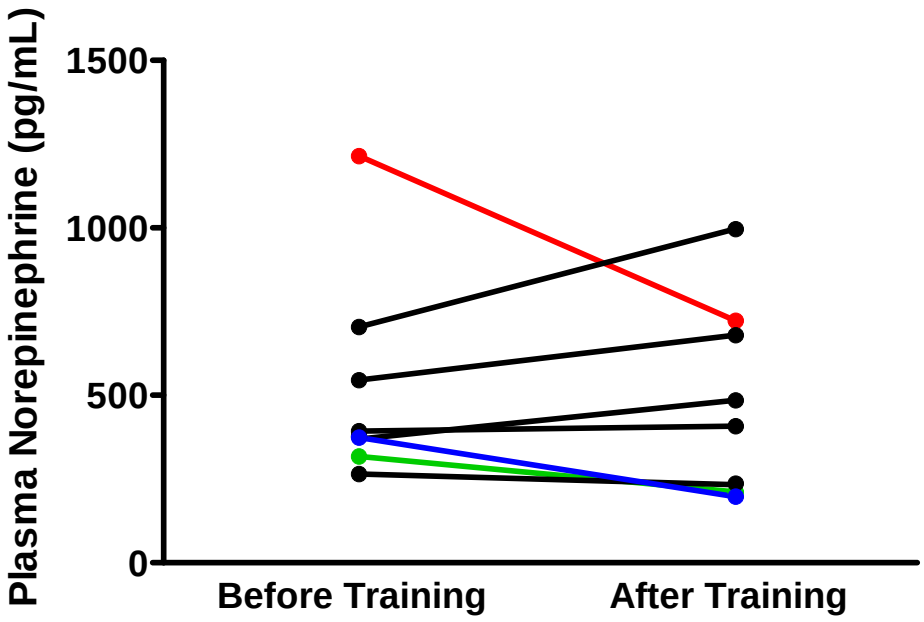


Figure 44. Individual plasma norepinephrine level before and after biofeedback training.

Three patients (highlighted in red, blue and green) had decreased levels of plasma norepinephrine following biofeedback training.

Table XIV. Detailed Patient Demographics for Biological Data

Status/Diagnosis	*Age*	*Sex*	*Race*	*LVEF*
NF	71	M	W	NA
NF	53	F	B	NA
NF	44	F	W	57
NF	18	M	W	"depressed EF"
NF	20	M	W	57
NF	61	M	NA	NA
NF	60	F	W	65
NF	16	F	W	NA
NF	59	M	W	NA
NF	NA	NA	NA	NA
NF	67	F	W	65
NF	54	M	W	63
F	66	M	O	15
F	61	M	W	10
F	58	M	W	20
F	60	F	W	10
F	66	M	W	NA
F	65	M	H	15
F	61	M	W	25
F	63	F	B	10
F	44	M	B	50
F	50	M	W	20
F	46	M	B	25
F	26	M	W	10
F + LVAD	62	M	W	13
F + LVAD	61	F	B	NA
F + LVAD	59	M	W	20
F + LVAD	52	M	W	NA
F + LVAD	27	M	W	5
F + LVAD	58	M	W	10
F + LVAD	64	M	W	15
F + LVAD	57	M	W	10
F + LVAD	51	M	W	15
F + LVAD	44	M	W	NA
F + LVAD	42	M	W	10
F + LVAD	64	M	W	10
F + BF	58	M	W	25
F + BF	58	M	W	15
F + BF	60	M	W	15
F + BF	53	F	B	15
F + BF	66	M	W	10
F + BF	46	M	W	60
F + BF	21	M	H	10
F + BF	65	M	W	20
F + BF	62	M	B	10
F + BF	66	M	W	15
F + BF	58	M	W	15
Patients who were added to Muscle Function and Western Blot Analyses				

Table XV. Summarized Patient Demographics for Biological Data

Group	*Age*	*Sex*	*Race*	*LVEF*	*Diagnosis*
Donor	48	6 M 5 F 1 UNK	9 W 1 B 2 UNK	61	-
Failing	56	10 M 2 F	7 W 3 B 1 H 1 "other"	19	8 DCM 4 ICM
LVAD	53	11 M 1 F	11 W 1 B	12	7 DCM 5 ICM
Biofeedback	56	10 M 1 F	8 W 2 B 1 H	19	7 DCM 3 ICM 1 CONG

M = Male; F = Female; UNK = Unknown; W = White; B = Black; H = Hispanic; DCM = Dilated Cardiomyopathy; ICM = Ischemic Cardiomyopathy; CONG = Congenital

manipulation (adding isoproterenol) and not to underlying differences in muscle function, contractile parameters were measured at baseline, and they can be found in **Table XVI**. No significant differences were found among groups for any of the six parameters.

These same six contractile parameters were also measured following the addition of ISO, and results were compared among groups as a percent change from baseline. Data from the F + BF group were also analyzed on an individual patient basis. **Figure 45** shows that there were no significant differences among groups with respect to the resting tension response (RT) ($p = 0.06$), and RT for 9 out of 11 patients in the F + BF group is at or approaching non-failing levels.

Developed tension (DT) results are depicted in **Figure 46**, and as we expected, muscles from failing hearts improved contraction less than muscles from non-failing hearts in response to beta-adrenergic stimulation ($p < 0.01$), and this response recovered in the F + LVAD group. Although not to the same extent as muscles from the F + LVAD group, the F + BF muscles also recovered such that there was no significant difference between the F + BF group and the non-failing group. On an individual patient basis, three patients in the F + BF showed a developed tension response at the non-failing level (IP 4, IP 6, and IP 8), and five patients' DT response fell somewhere in between failure and non-failure (IP 2, IP 3, IP 15, IP 20 and IP 21). The last three patients in the F + BF group had a DT response to isoproterenol that was at the failing level (IP 1, IP 11 and OP 2).

Figure 47 shows the time to peak tension (TPT) results. No significant differences were found among groups ($p = 0.70$), and although the non-failing and failing means are so similar (-23.4% and -22.0%, respectively), the individual patient data from

Table XVI. Contractile Parameters at Baseline

Contractile Parameters	NF	Failing	F + LVAD	F + BF
RT (g/mm^2)	3.11 ± 0.38	2.75 ± 0.30	2.51 ± 0.33	2.04 ± 0.15
DT (g/mm^2)	1.12 ± 0.15	0.99 ± 0.15	1.69 ± 0.25	0.72 ± 0.15
TPT (sec)	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.17 ± 0.00
THR (sec)	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.00
+dT/dt ($g/sec/mm^2$)	9.11 ± 1.26	10.41 ± 1.31	11.06 ± 1.40	7.70 ± 1.35
-dT/dt ($g/sec/mm^2$)	7.66 ± 1.25	8.85 ± 1.19	9.76 ± 1.19	6.28 ± 1.33

NF = Non-failing; F + LVAD = Failing + Left Ventricular Assist Device; F + BF = Failing + Biofeedback; RT = Resting Tension; DT = Developed Tension; TPT = Time to Peak Tension; THR = Time to Half Relaxation; +dT/dt = Peak Rate of Tension Rise; -dT/dt = Peak Rate of Tension Fall

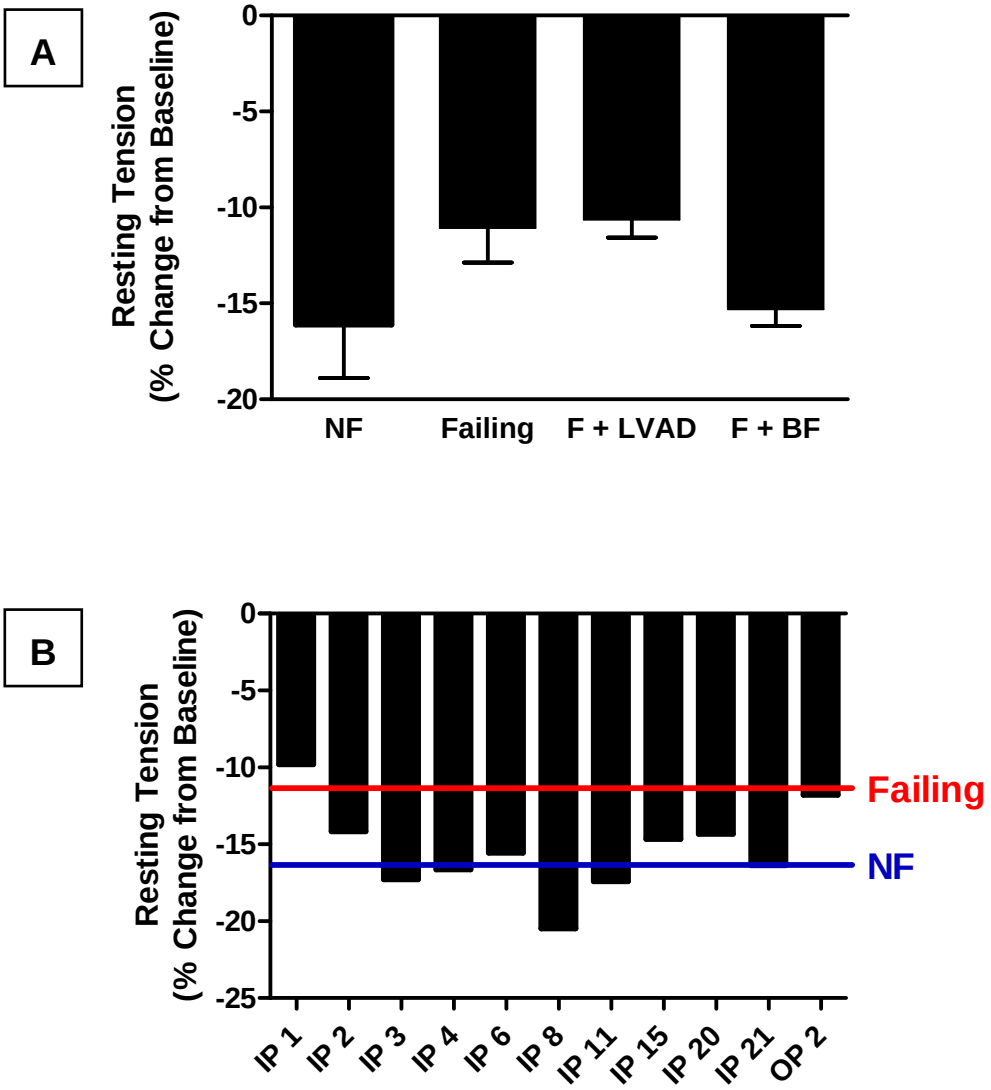


Figure 45. Resting tension following addition of isoproterenol.

(A) No significant differences were found among groups for the resting tension response to isoproterenol ($p = 0.06$), and (B) the majority of patients in the F + BF group had muscles with resting tension levels at or near non-failing muscles.

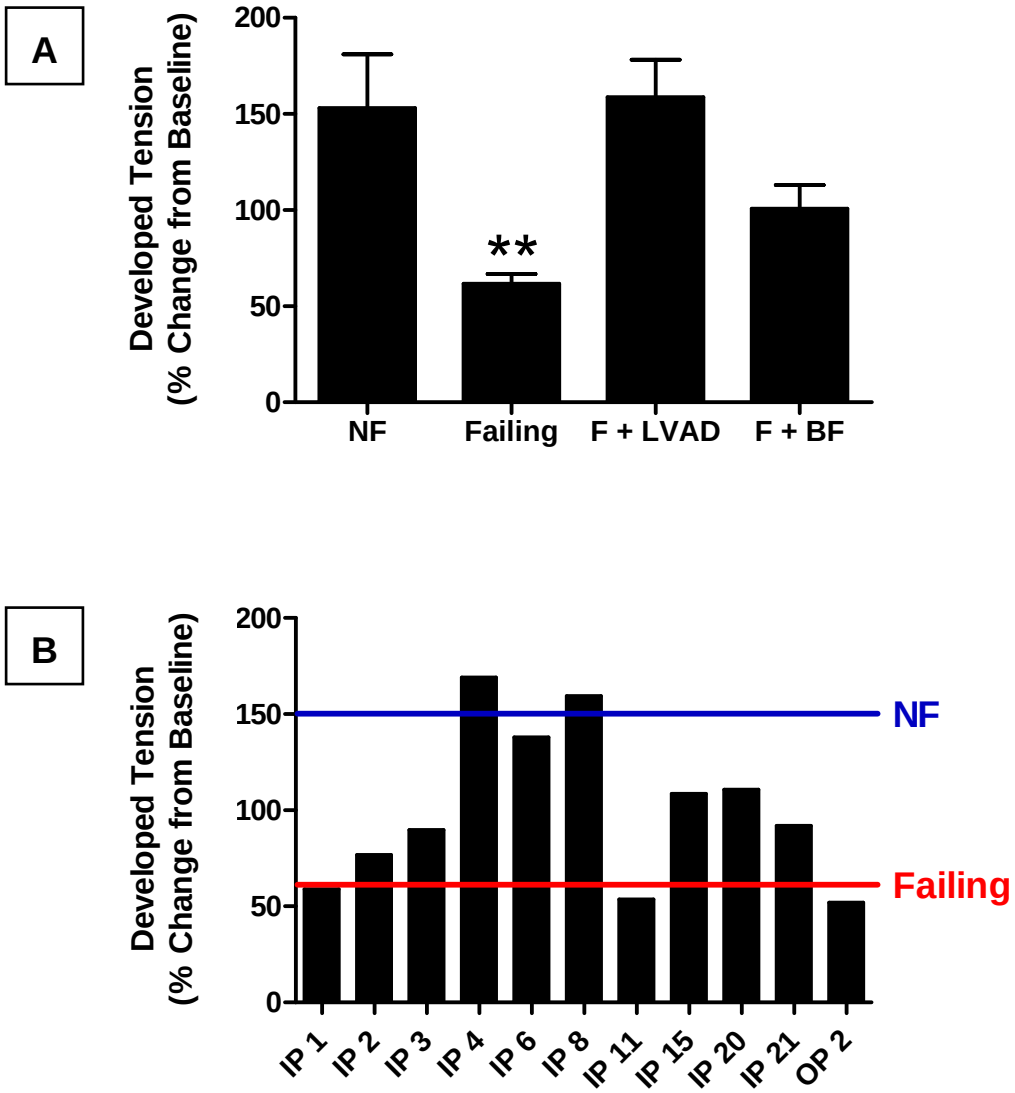


Figure 46. Developed tension following addition of isoproterenol.

(A) Muscles taken from failing hearts contracted significantly less than muscles taken from non-failing hearts in response to isoproterenol ($p < 0.05$). (B) Muscles taken from three patients in the F + BF group contracted at non-failing levels in response to isoproterenol, and five others contracted more than the average of muscles in the failing group.

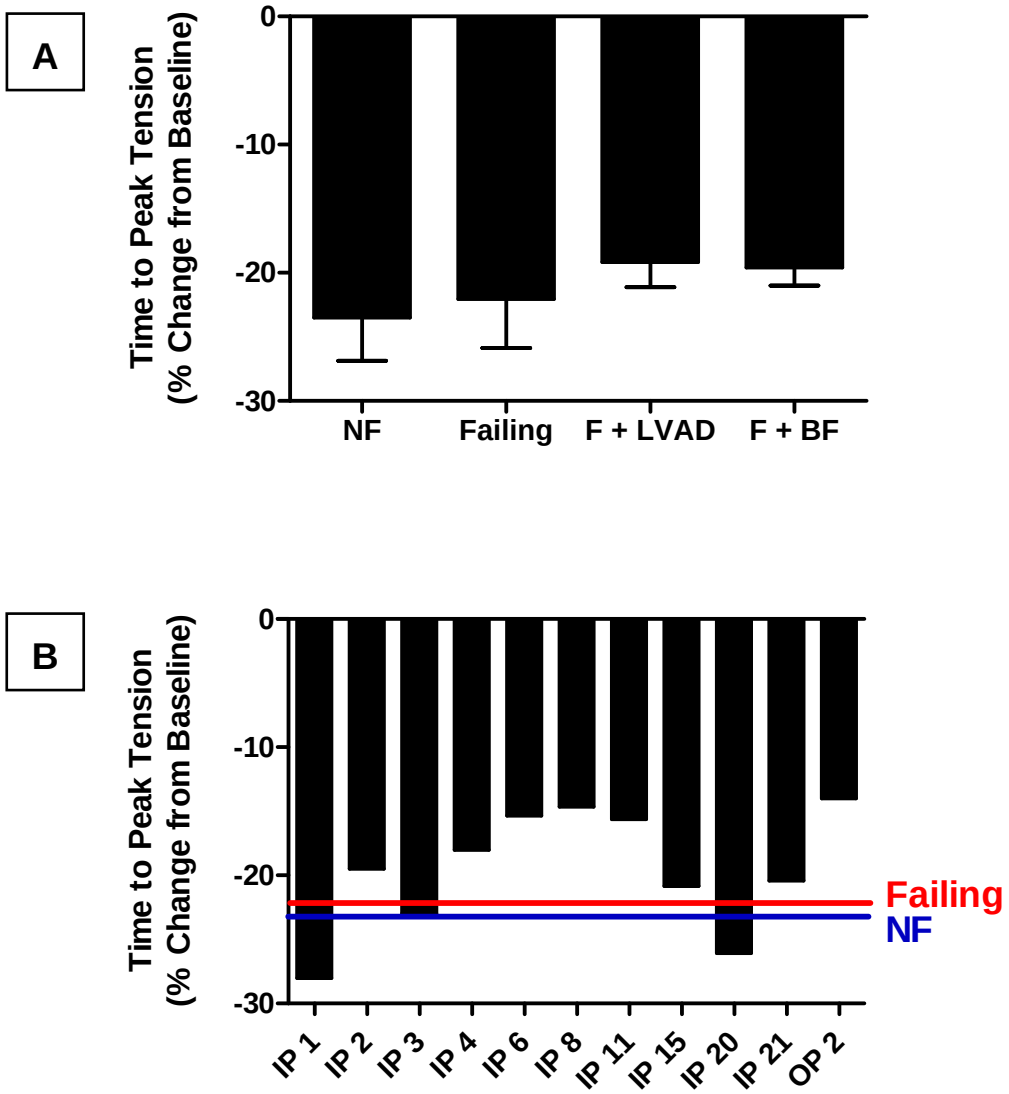


Figure 47. Time to peak tension following addition of isoproterenol.

(A) No significant differences were found among groups for time to peak tension ($p = 0.70$), and (B) the majority of patients in the F + BF group had muscles with time to peak tension levels at or near failing muscles.

the F + BF group showed that three patients have TPT responses that are at or surpass the non-failing level.

The results of time to half relaxation (THR) analyses did not show any significant differences among groups ($p = 0.11$) as shown in **Figure 48**. The individual analysis of patients in the F + BF group showed that the THR response for most of the muscles was like that of muscles taken from non-failing hearts, but again, the means from the non-failing (-30.3%) and failing (-33.4%) groups are very similar.

Figure 49 shows that peak rate of tension rise (+dT/dt) was lower in the failing group relative to muscles taken from non-failing hearts ($p < 0.01$). This response was not seen in the F + LVAD or F + BF groups, both of which did not differ significantly from the non-failing group. Also, the three patients in the F + BF group who had a developed tension response to isoproterenol that was equivalent to that of muscles from non-failing hearts (IP 4, IP 6 and IP 8) were also the three patients whose peak rate of tension rise response was at or above the non-failing level.

The peak rate of tension fall (-dT/dt) results were very much like peak rate of tension rise. As **Figure 50** shows, peak rate of tension fall was lower in the failing group relative to the non-failing group ($p < 0.01$). Both the F + LVAD group and the F + BF group were not significantly different from non-failing hearts. On an individual patients basis, muscles taken from hearts of F + BF inpatients 4, 6 and 8 again looked like muscles from the non-failing group with respect to –dT/dt.

Beta-Adrenergic Receptors

To further explore the sympathetic nervous system pathway, beta-adrenergic

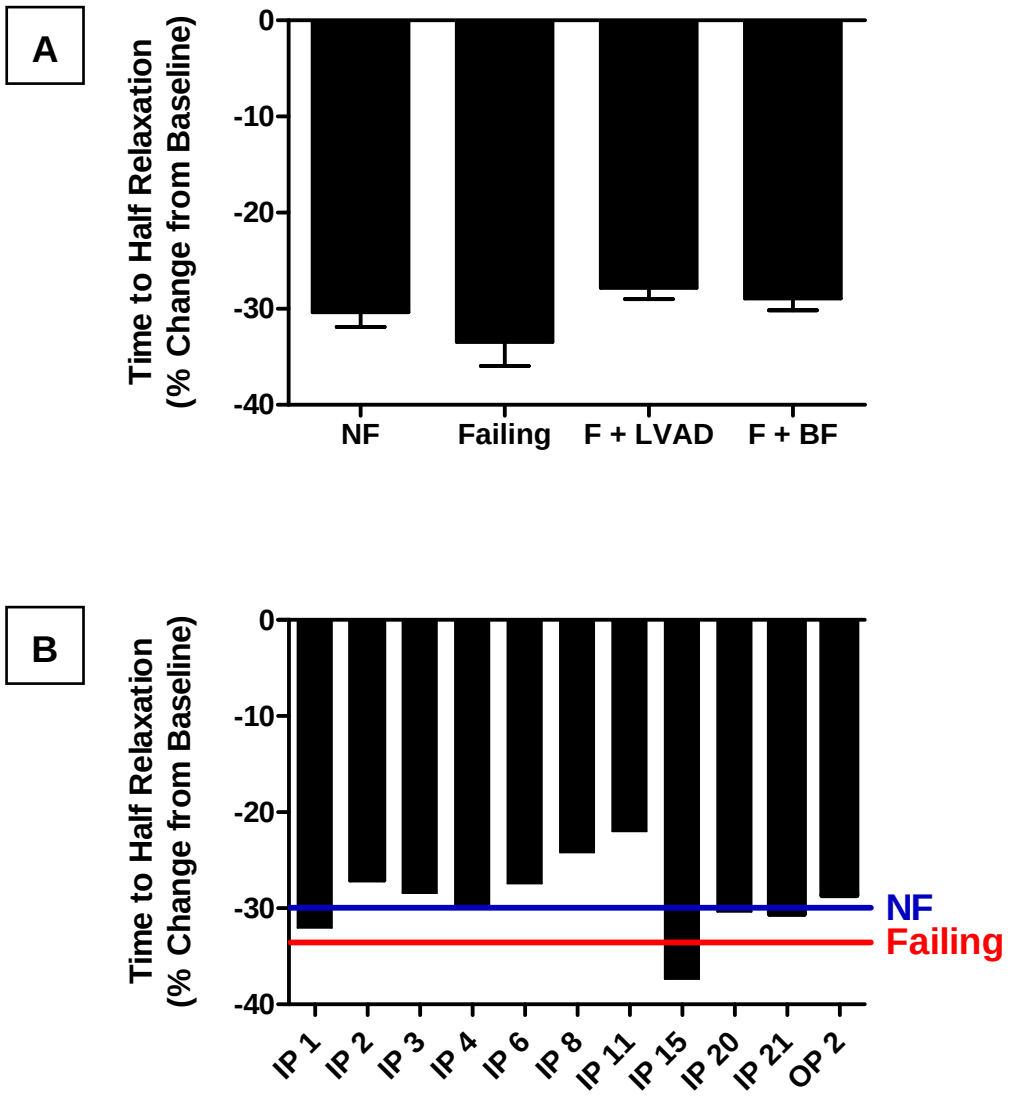


Figure 48. Time to peak half relaxation following addition of isoproterenol.

(A) No significant differences were found among groups for time to half relaxation ($p = 0.11$), and (B) the majority of patients in the F + BF group had muscles with time to half relaxation levels at or near non-failing muscles.

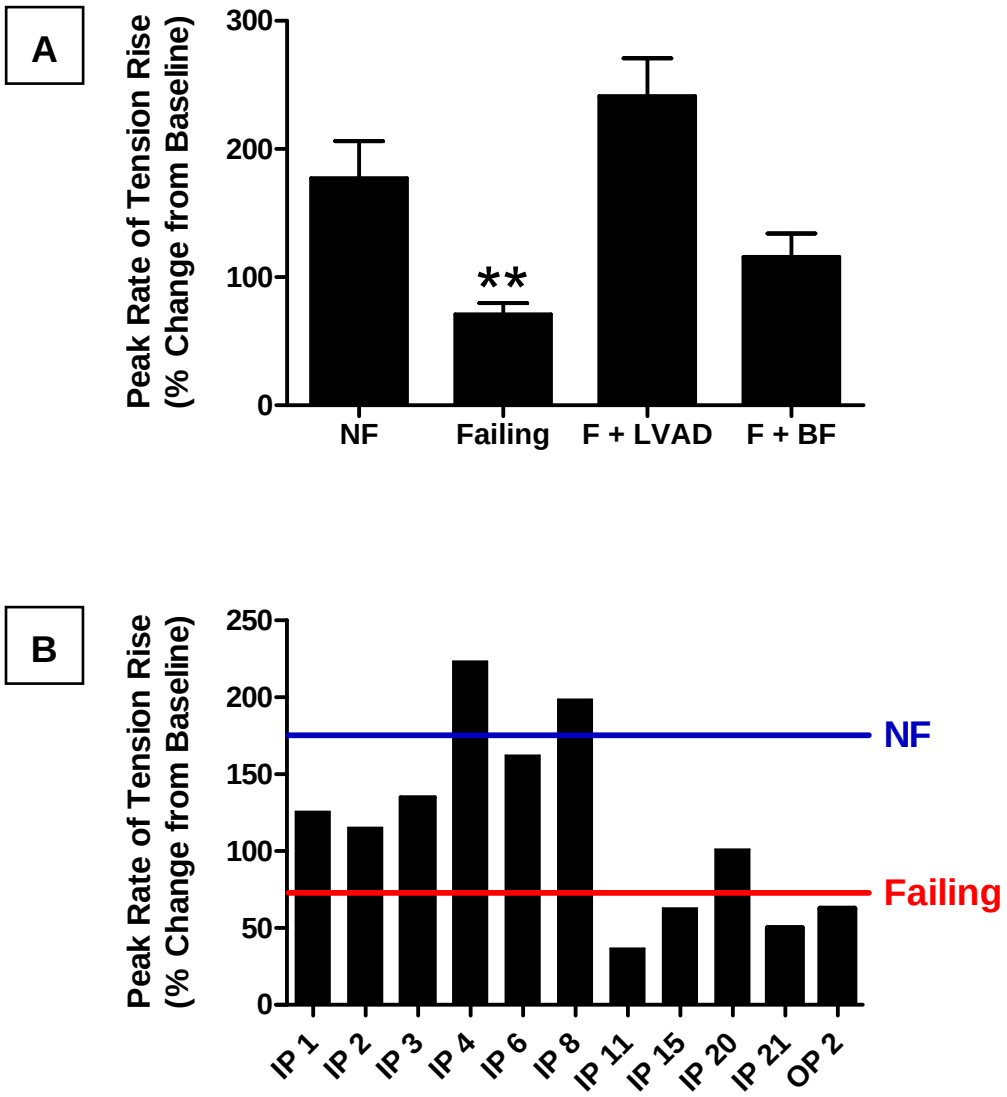


Figure 49. Peak rate of tension rise following addition of isoproterenol.

(A) Muscles taken from failing hearts had a lower peak rate of tension rise in response to isoproterenol ($p < 0.01$) relative to non-failing hearts. (B) Muscles taken from three patients in the F + BF group had peak rate of tension rise responses at the level of non-failing muscles. These were the same three patients whose developed tension response was also at or above the non-failing level.

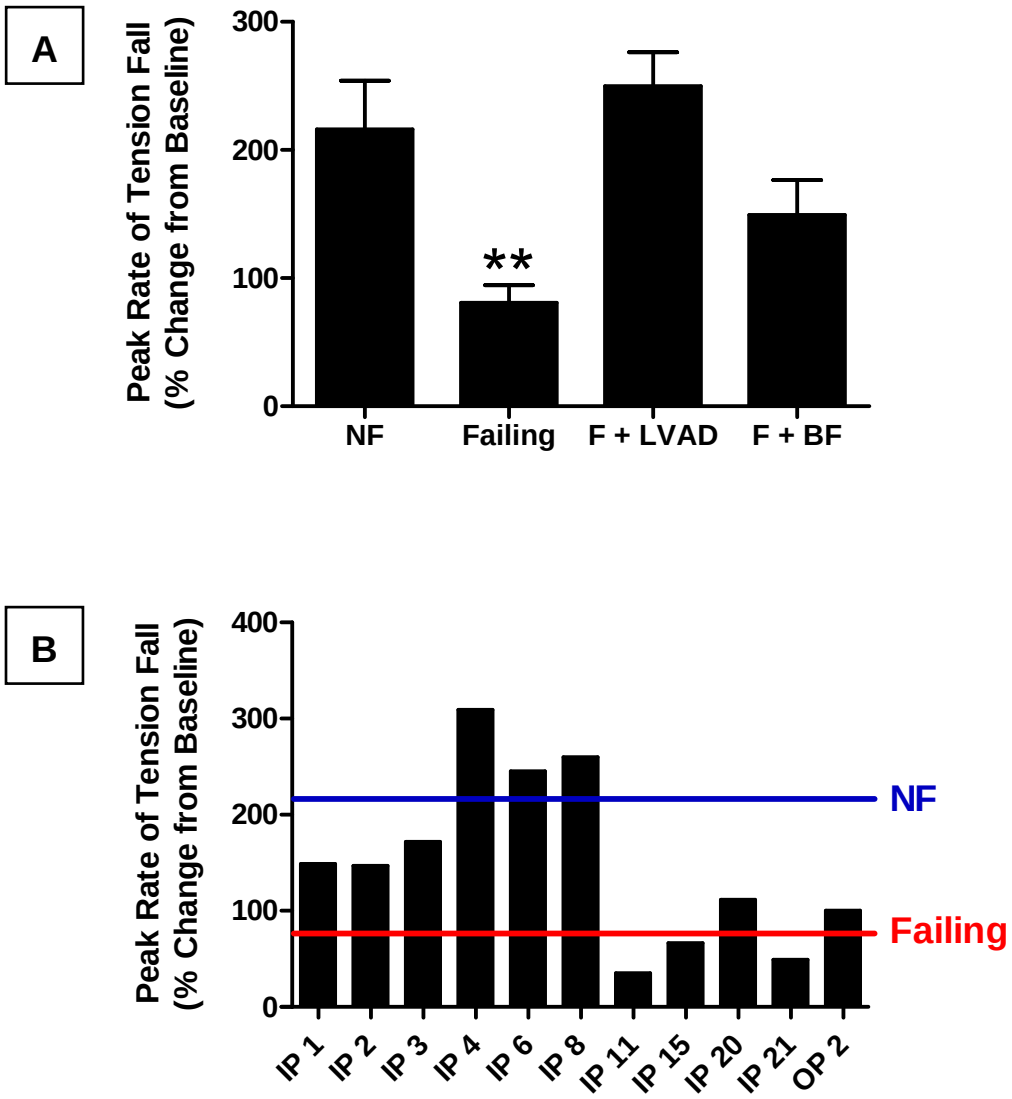


Figure 50. Peak rate of tension fall following addition of isoproterenol.

(A) Muscles taken from failing hearts had a lower peak rate of tension fall in response to isoproterenol ($p < 0.01$) relative to non-failing hearts. (B) Muscles taken from three patients in the F + BF group had peak rate of tension fall responses at the level of non-failing muscles. These were the same three patients whose developed tension and peak rate of tension rise responses were also at or above the non-failing level.

receptors were measured among non-failing (NF), failing, F + LVAD and F + BF groups. Once again, our group of interest, the F + BF group, was also analyzed on an individual patient basis. As shown in **Figure 51**, beta receptor density was significantly lower in failing hearts ($p < 0.05$) and recovered in failing hearts with LVAD support. Beta receptor density in the F + BF group did not recover like the F + LVAD group, but rather was significantly less than that of the non-failing hearts ($p < 0.05$). Individually, three patients in the F + BF group showed beta receptor recovery at the non-failing level (IP 1, IP 8 and IP 15). Only one of these patients (IP 8) was one of the patients whose developed tension response to isoproterenol was also at the non-failing level.

Binding affinity for beta-adrenergic receptors was also analyzed among groups by measuring the dissociation constant (Kd), and **Figure 52** shows that no significant differences were found ($p = 0.06$). The overall p-value was very close to being significant, and groups with the lowest and highest mean Kd were the F + LVAD (31.4 ± 12.9 pM) and F + BF group (52.3 ± 21.8), respectively. Individual patient analysis for the F + BF group showed that half of the patients had a Kd that was 40pM or below, and the other half of the patients had a Kd that was much higher, at 60pM or above.

Muscarinic Receptors

To analyze the contribution of the parasympathetic nervous system, muscarinic receptor density and Kd were also measured. As shown in **Figure 53**, muscarinic receptor density was significantly higher in the failing ($p < 0.01$) and F + LVAD ($p < 0.001$) groups relative to NF hearts. There was no difference between the NF and F + BF groups. With the exception of one statistical outlier, highlighted in white, most patients

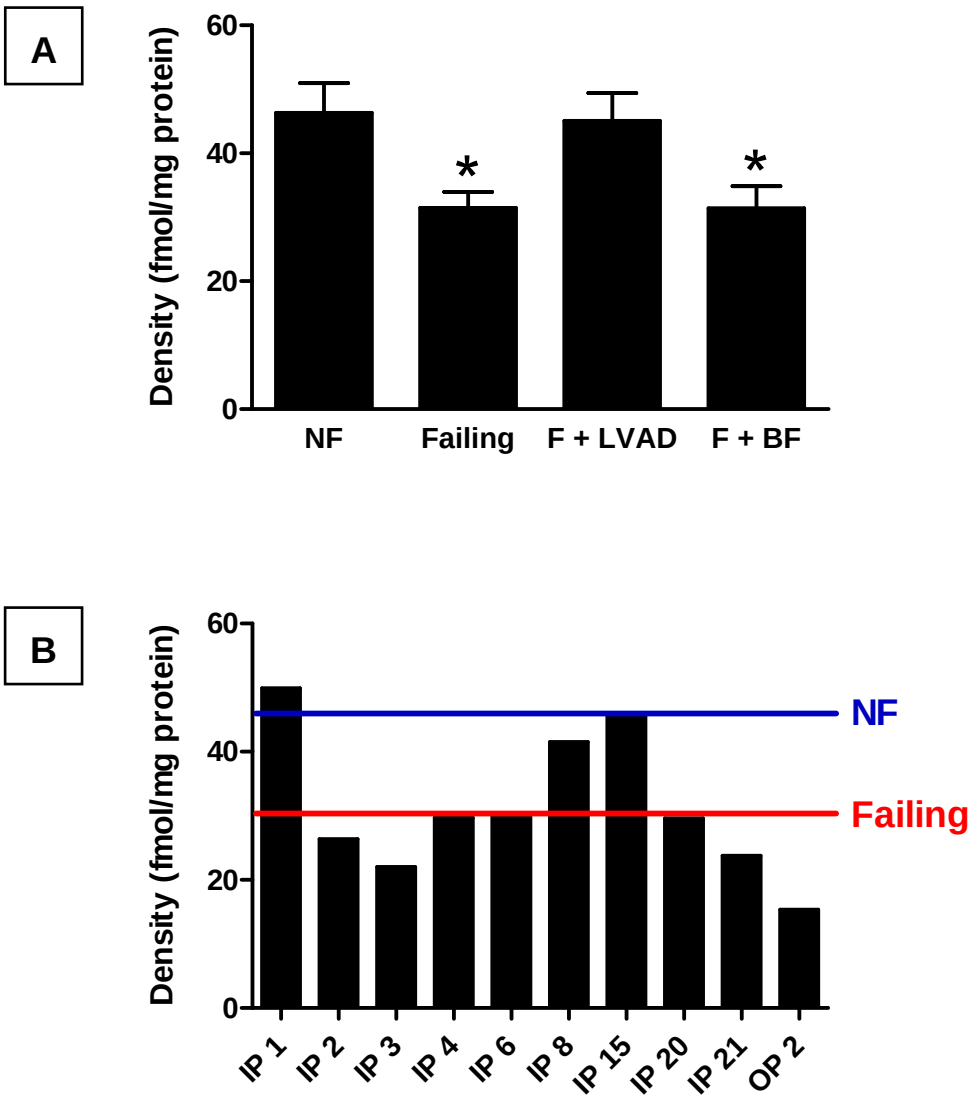


Figure 51. Beta-adrenergic receptor density.

(A) The density of beta-adrenergic receptors was significantly lower in the failing and F + BF groups compared to beta receptor density in non-failing hearts ($p < 0.05$). (B) Three patients in the F + BF group showed beta-adrenergic receptor densities at the non-failing level. Only one of these patients was also one whose developed tension response to isoproterenol was also at the non-failing level.

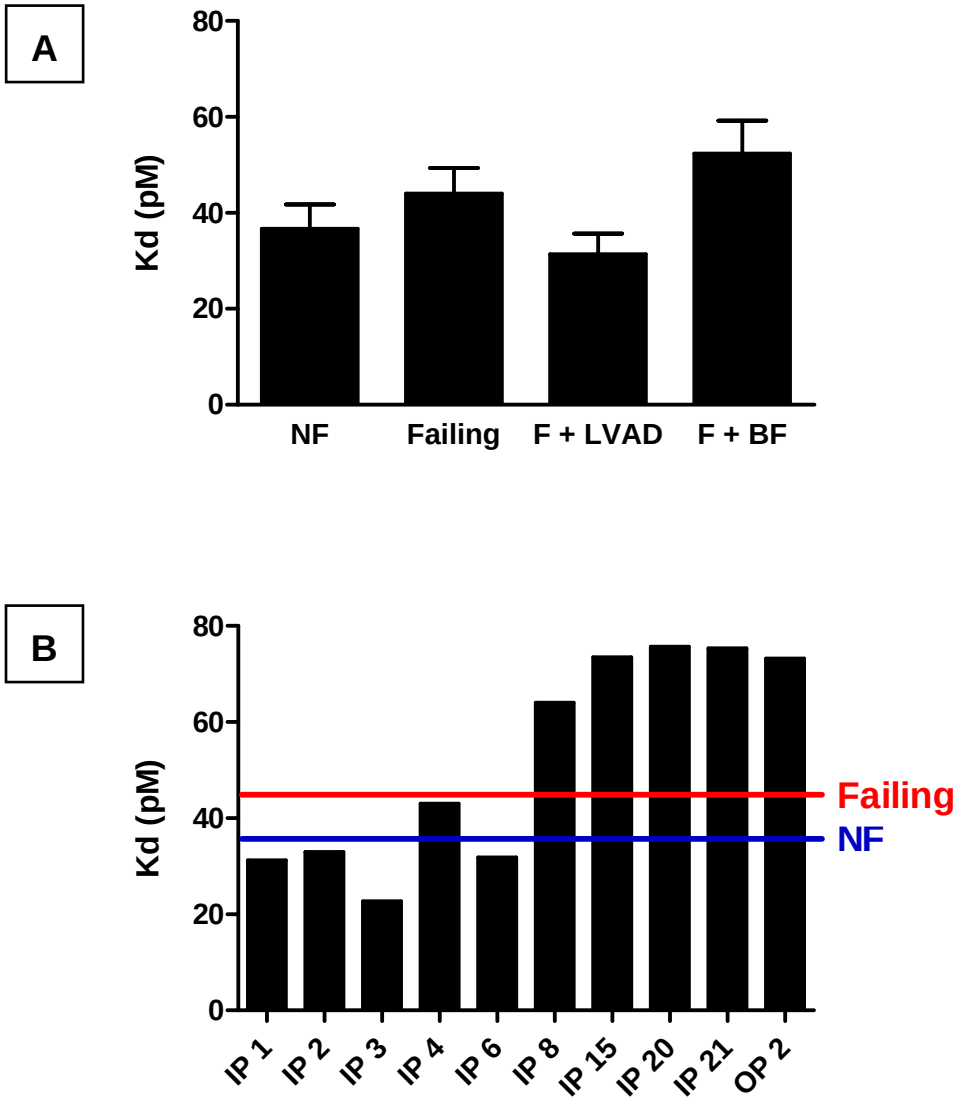


Figure 52. Binding affinity for beta-adrenergic receptors.

(A) Beta-adrenergic receptor Kd was not significantly different among groups ($p = 0.06$). (B) Half of the patients in the F + BF group had Kd's that were much higher than the other half of patients.

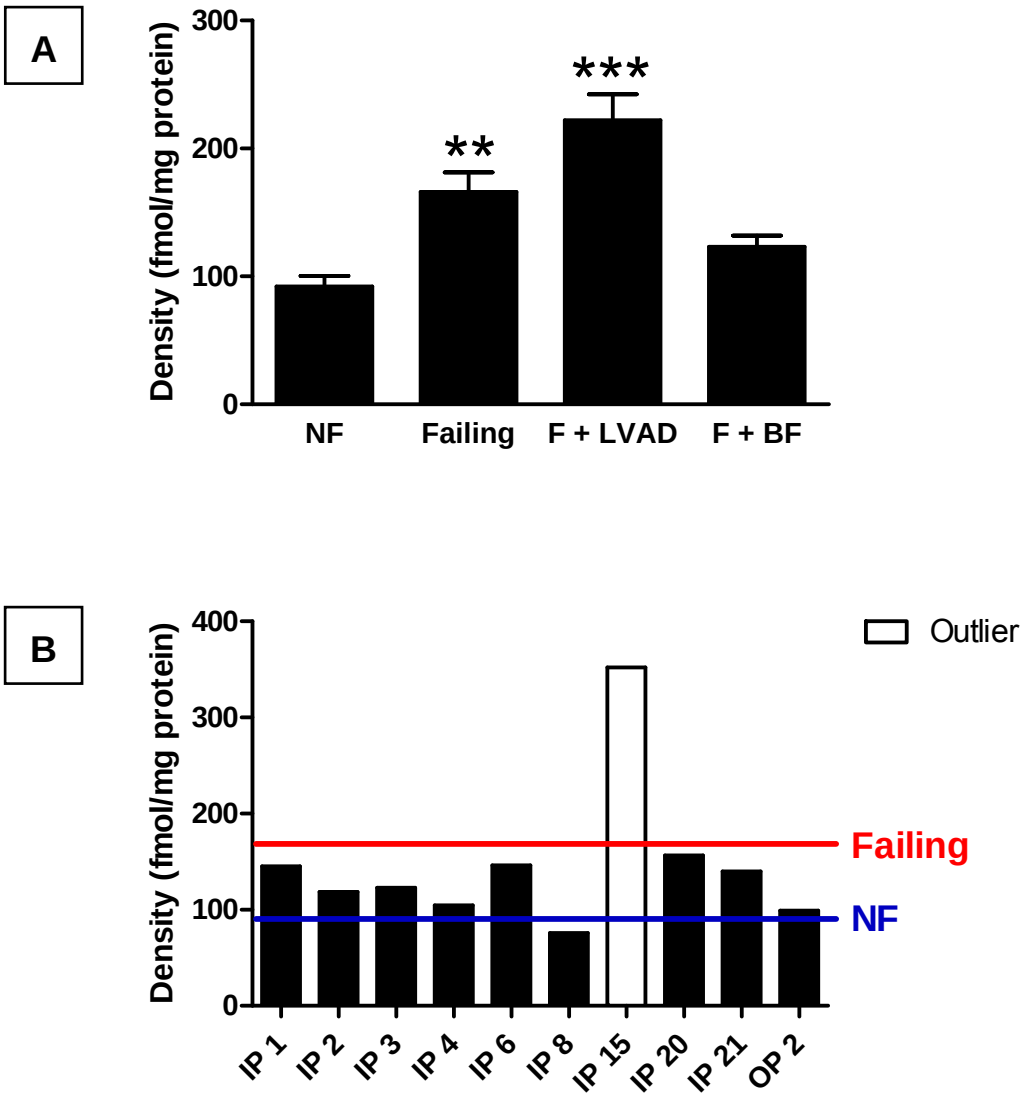


Figure 53. Muscarinic receptor density.

(A) Muscarinic receptor density was significantly higher in the failing ($p < 0.01$) and F + LVAD ($p < 0.001$) groups relative to non-failing hearts. The F + BF group was not significantly different from the non-failing group. (B) Most patients in the F + BF group had muscarinic receptor densities at or just above the non-failing level. IP 15 is an outlier.

in the F + BF group had muscarinic receptor densities at or just above the non-failing level.

Figure 54 shows that no significant differences were found among groups with respect to binding affinity for muscarinic receptors ($p = 0.07$). This Kd data almost did reach statistical significance, with the highest and lowest mean Kd's coming from the non-failing (119.9 ± 33.8 pM) and F + LVAD (230.1 ± 112.9 pM) groups. In the F + BF group, most muscarinic receptor Kd's were at or just below the mean of the non-failing group, with the exception of two much higher values and one outlier Kd of 405.6 pM.

Calcium-Cycling Proteins

With calcium being a vital component of muscle contraction, proteins that help to cycle calcium in and out of the sarcoplasmic reticulum and in and out of the cell itself were measured. In all cases, calcium-cycling proteins were normalized to calsequestrin, a protein that has been shown not to change in heart failure.[27,53,83]

Figure 55 shows that no significant differences were found among groups with respect to the sarcoendoplasmic reticulum calcium ATP-ase (SERCA) protein ($p = 0.62$). Variable levels of SERCA protein were found in the F + BF group.

No significant differences among groups were found in sodium-calcium exchanger (NCX) protein levels ($p = 0.73$), as shown in **Figure 56**, and the F + BF group presented with variable NCX levels, most of which were at or above the non-failing average.

With average values of 0.47 ± 0.19, 0.27 ± 0.14 and 0.36 ± 0.22 relative densitometric units (RDU), the failing, F + LVAD and F + BF groups, respectively, had

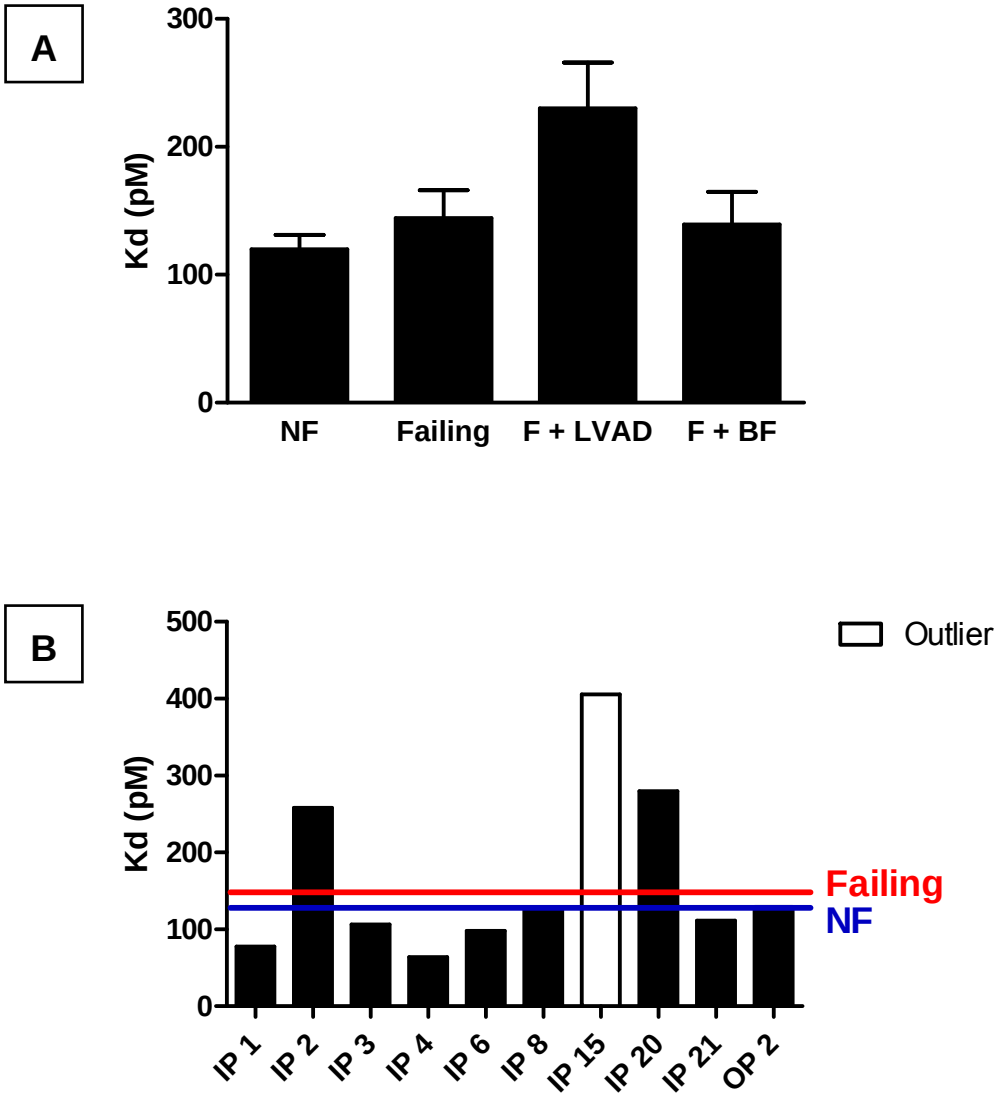


Figure 54. Binding affinity for muscarinic receptors.

(A) Muscarinic receptor Kd was not significantly different among groups ($p = 0.07$). (B) Most patients in the F + BF group had Kd's that were at the non-failing level. IP 15 is an outlier.

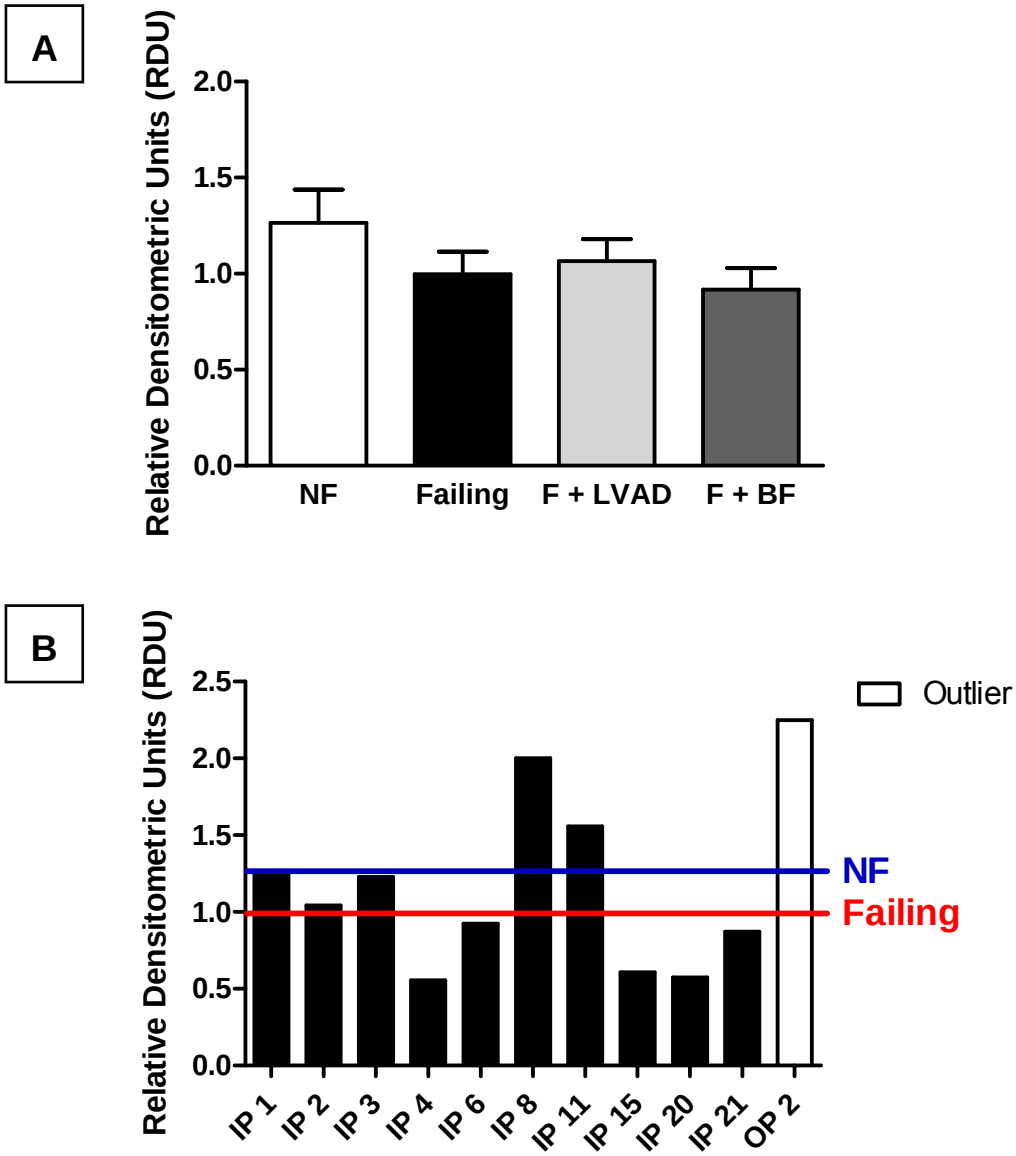


Figure 55. SERCA/CALQ.

(A) There were no significant differences in SERCA protein among groups (p = 0.62). (B) SERCA protein levels in the F + BF group were variable. OP 2 is an outlier.

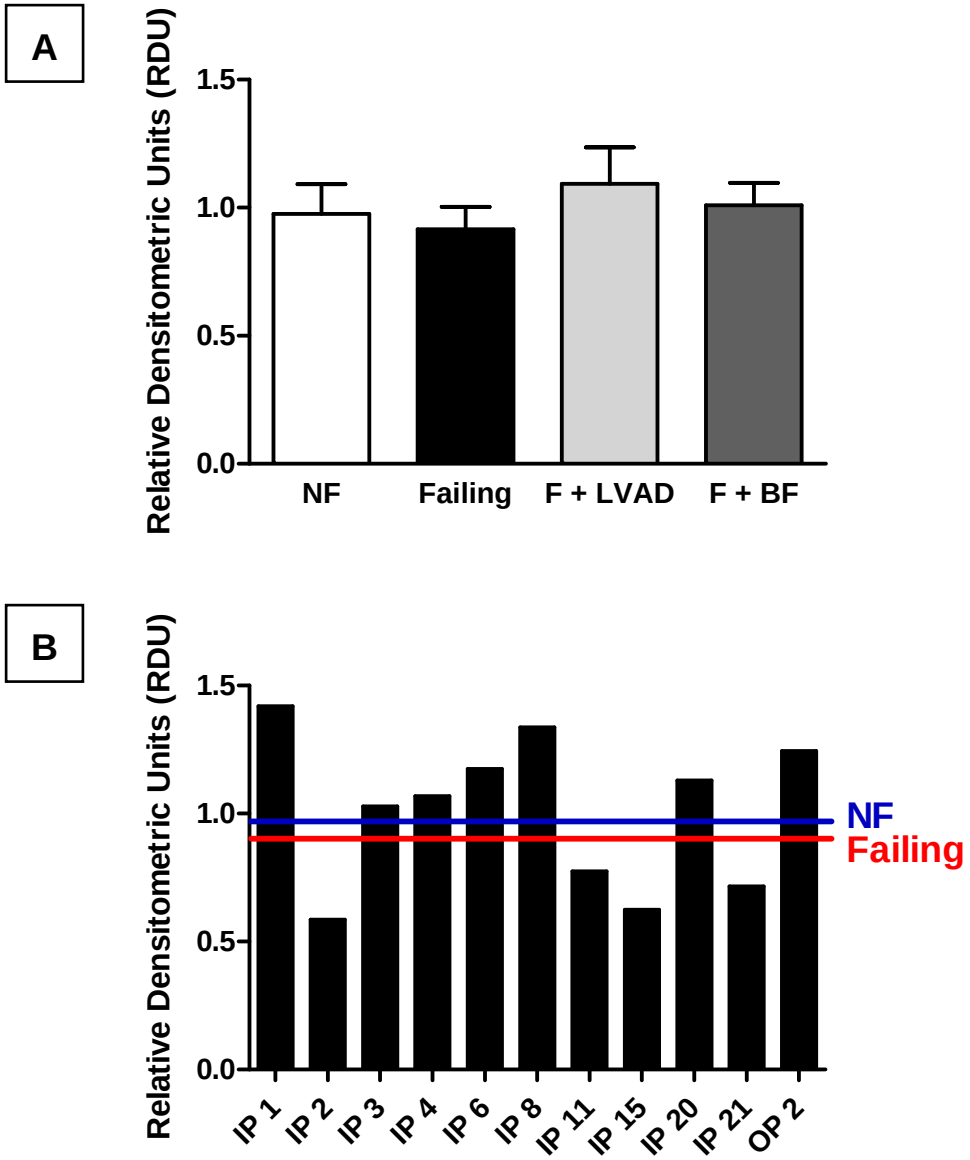


Figure 56. NCX/CALQ.

(A) There were no significant differences in NCX protein among groups (p = 0.73). (B) NCX protein levels in the F + BF group were variable, however most were at or above the non-failing level.

significantly lower ryanodine receptor (RYR) protein levels than the non-failing group (0.75 ± 0.26 RDU; overall $p < 0.001$). **Figure 57** highlights these differences and shows the individual variability in the F + BF group. One F + BF patient had RYR levels at the non-failing level, and two others were approaching the non-failing average.

3.7 Correlation Data

In order to determine whether patients who were successful with biofeedback were the same patients who showed changes in the biology of the heart, each psychophysiologic variable that changed following biofeedback training was correlated with each changing biological variable. These correlation analyses were performed using six patients (IP 1, IP 3, IP 4, IP 8, IP 11, IP 15). There were eleven patients in the biofeedback group on whom biological data were collected (Tables XIV and XV), but only six of these patients also had before and after biofeedback training data (these six patients completed all eight sessions of biofeedback) (Table XIII). Linear regression analyses were also performed, and the results (p-values) are summarized in **Table XVII.** No statistically significant relationships were found.

The two correlations highlighted in bold boxes in Table XVII were not significant ($p > 0.05$), however both included a point outside the 95% confidence interval. This point was from the same patient in each relationship, IP 11. Because IP 11 is the only patient with congenital heart disease in the study, these two relationships were analyzed both with and without IP 11, and removing this patient created highly significant relationships between these two sets of variables.

Figure 58 shows the relationship between average respiration rate during the first

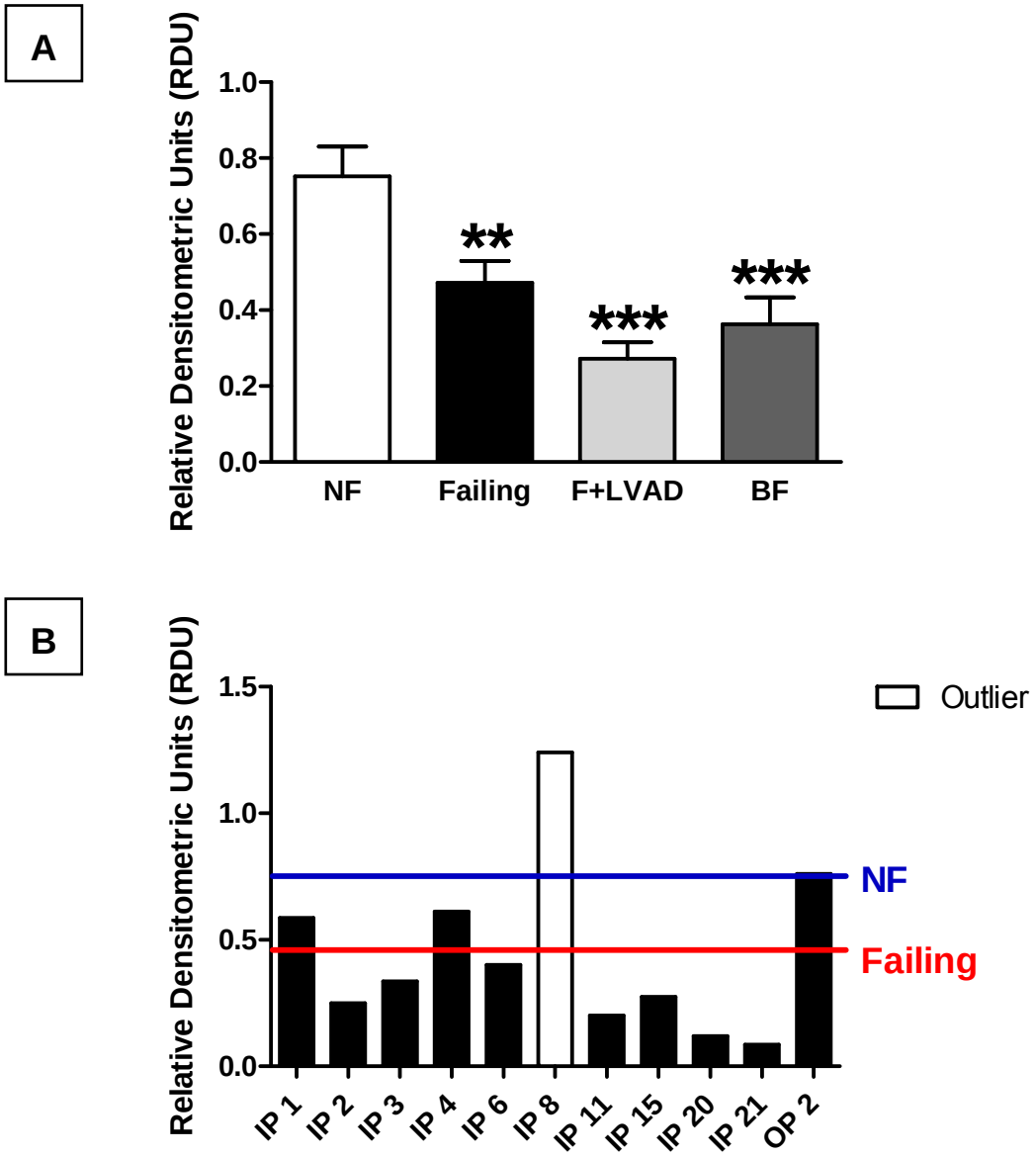


Figure 57. RYR/CALQ.

(A) RYR levels were significantly lower in the failing ($p < 0.01$), F + LVAD ($p < 0.001$) and F + BF ($p < 0.001$) groups relative to the non-failing group. (B) RYR protein levels in the F + BF group were variable, and most were below the failing level. Three patients, however, showed RYR levels at or approaching the non-failing level. IP 8 is an outlier.

Table XVII. Biofeedback vs. Biology Correlation Data

	DT (ISO)	B-AR	MR	RYR
Resp Rate (in Session 8)	0.98	0.71	0.79	**0.17**
Resp Rate (% Change)	**0.17**	0.47	0.24	0.85
SDNN (in Session 8)	0.30	0.96	0.30	0.87
SDNN (% Change)	0.45	0.34	0.23	0.37
Agg. CV React (% Change)	0.91	0.61	0.11	0.08
Agg. CV Recover (% Change)	0.22	0.50	0.26	0.72
Homework	0.87	0.16	0.56	0.99

From top to bottom, biofeedback variables analyzed include: Average respiration rate during the first 5-minute self-relaxation of session 8; Respiration rate as a percent change from the first 5-minute self-relaxation of session 1 to the first 5-minute self-relaxation of session 8; Average SDNN during the first 5-minute self-relaxation of session 8; SDNN as a percent change from the first 5-minute self-relaxation of session 1 to the first 5-minute self-relaxation of session 8; Aggregate cardiovascular reactivity as a percent change difference (session 8 minus session 1); Aggregate cardiovascular recovery as a percent change difference (session 8 minus session 1); Number of homework sheets turned in.

DT (ISO) = % change in developed tension following the addition of isoproterenol; B-AR = Beta-adrenergic receptor density; MR = Muscarinic receptor density; RYR = Ryanodine receptor protein expression.

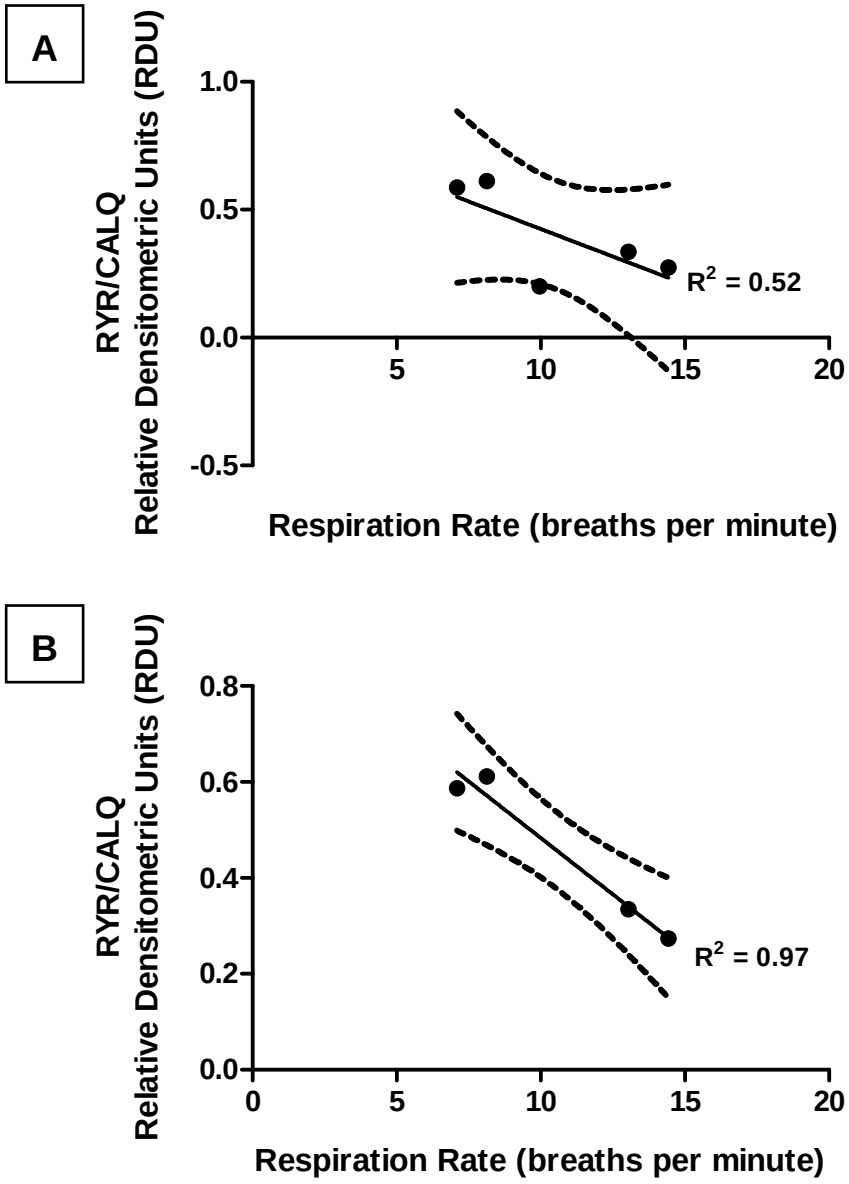


Figure 58. Relationship between respiration rate and ryanodine receptor protein expression.

(A) WITH IP 11 – There is no significant relationship between average respiration rate during the first 5-minute self-relaxation of session 8 and ryanodine receptor protein expression ($p = 0.17$; $R^2 = 0.52$). (B) WITHOUT IP 11 – Patients who were breathing at lower rates in session 8 had greater expression of ryanodine receptor protein ($p = 0.02$; $R^2 = 0.97$).

5-minute self-relaxation of session 8 and expression of the ryanodine receptor protein, both with and without IP 11. While no significant relationship existed in the presence of IP 11 ($p = 0.17$; $R^2 = 0.52$), removing the IP 11 data point created a significant correlation in which patients who were breathing at lower rates following biofeedback training had greater expression of ryanodine receptor protein ($p = 0.02$; $R^2 = 0.97$).

A significant relationship between percent change in respiration rate and developed tension in response to isoproterenol also emerged when IP 11 was removed, as shown in **Figure 59** ($p = 0.01$; $R^2 = 0.90$). Specifically, patients who lowered their respiration the most (negative percent change) had greater developed tension responses to isoproterenol.

In addition to relationships between biofeedback and biological data, correlations between transplant wait time following biofeedback training and the biological variables analyzed above were also explored. **Table XVIII** displays the number of days patients waited for transplant following their participation in the biofeedback study, and **Table XVIIII** shows the correlation results (p-values). No significant relationships were found.

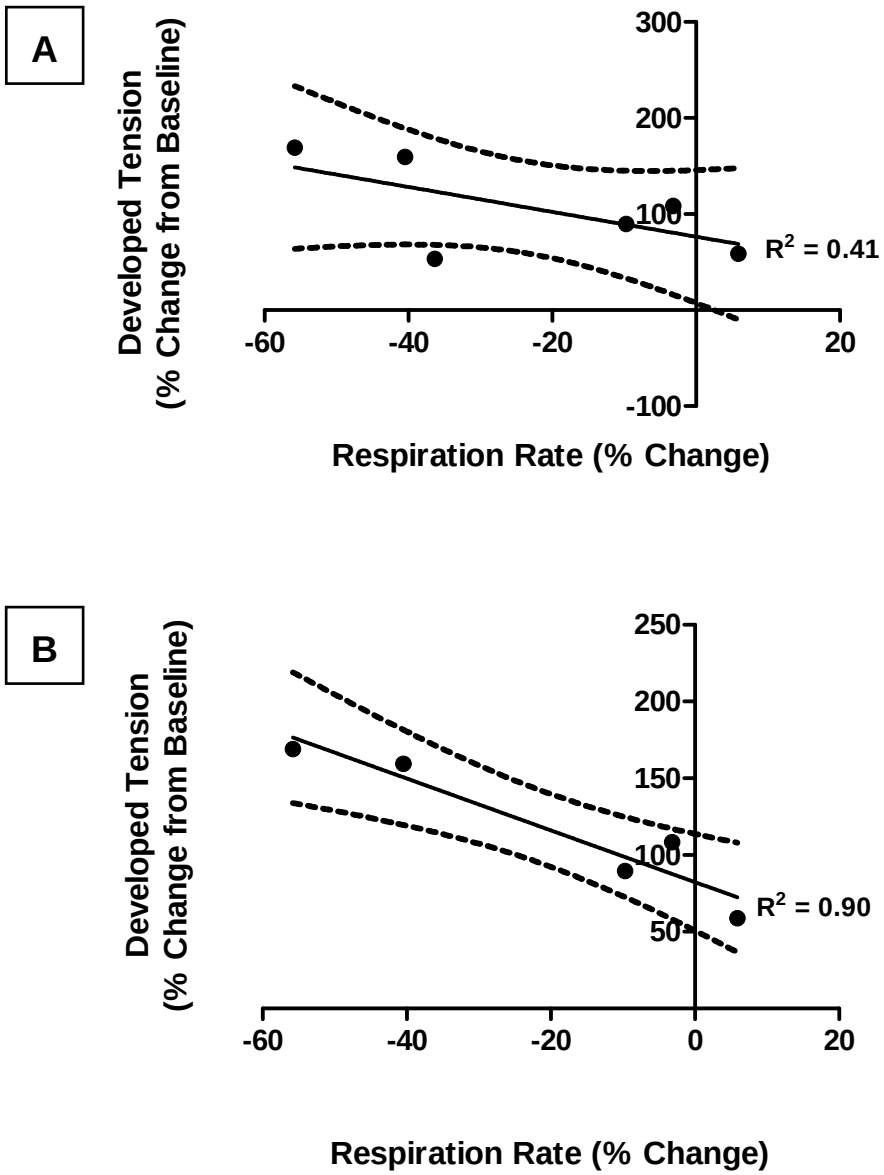


Figure 59. Relationship between respiration rate and developed tension response to isoproterenol.

(A) WITH IP 11 – There is no significant relationship between percent change in respiration rate and developed tension in response to isoproterenol ($p = 0.17$; $R^2 = 0.41$). (B) WITHOUT IP 11 – Patients who lowered their respiration rate the most had greater developed tension responses to isoproterenol ($p = 0.01$; $R^2 = 0.90$).

Table XVIII. Transplant Wait Time

Patient ID	BF ⟶ TX (days)
IP1	21
IP 3	37
IP 4	40
IP 8	7
IP 11	257
IP 15	230

Table XVIIII. Transplant Wait Time vs. Biology Correlation Data

	DT (ISO)	B-AR	MR	RYR
BF → Tx (days)	0.40	0.65	0.60	0.09

Data are displayed as p-values.

CHAPTER IV

DISCUSSION

Self-regulation techniques have been explored in the treatment of heart failure prior to this study. Mindfulness training has been shown to reduce anxiety and depression and to improve clinical symptoms in patients with heart failure.[106] Quality of life has been shown to improve following both relaxation training[21,124] and meditation[26], and meditation has also been shown to reduce circulating norepinephrine.[26]

4.1 Biofeedback in End-Stage Heart Failure Patients

Although the potential for other mind-body therapies to play a role in heart failure has been shown, there have only been a few studies exploring the efficacy of biofeedback training in patients with documented heart failure, and therefore the ability for heart failure patients to learn biofeedback was unknown.

Respiration Rate

Respiration rate was the first physiologic modality focused on in the biofeedback-

assisted stress management training protocol. Patients were taught to breathe from the diaphragm as opposed to the chest and were encouraged to breathe slowly and deeply. Over the course of the study, patients were able to progressively decrease their breathing rate, resulting in a significantly lower rate after only two training sessions. Before biofeedback training, patients' average breathing rate was 14.9 ± 2.9 breaths per minute, and after biofeedback training, average respiration rate decreased to 9.4 ± 2.7 breaths per minute. This result is similar to a study done by Bernardi and colleagues in 1998, however that study used diaphragmatic breathing training only, not biofeedback.

Bernardi showed that in nine heart failure patients practicing diaphragmatic breathing at home for one hour every day for a month, spontaneous breathing rate dropped from 13.4 ± 1.5 to 7.6 ± 1.9 breaths per minute.[7] While the absolute change in respiration rate is similar in our study and the Bernardi study (~ 5.5 breaths per minute), patients in the Bernardi study had lower breathing rates both before and after intervention. What was not reported in Bernardi's study, however, are the clinical demographics of these nine heart failure patients, and it is possible that our heart failure population (NYHA class III or IV, average LVEF of 23%) had more severe disease.

Other studies exploring the benefits of slower breathing in heart failure patients did not use biofeedback and did not report respiration rate before and after intervention, but showed that relative to a control group of heart failure patients, lowering respiration rate results in decreased dyspnea[121], improved exercise tolerance[6,121], and lower blood pressure[95].

Digital Peripheral Temperature

Digital peripheral temperature was measured in this study as an indirect correlate of peripheral vasoconstriction. When a person is relaxed, their blood vessels dilate, allowing more warm blood to pass through. Because biofeedback cannot directly measure blood vessel diameter, it measures finger temperature instead. This is because the fingers have a dense network of blood vessels with relatively little surrounding tissue. As a result, changes in temperature occur relatively more rapidly in the fingertips.[103]

We expected that biofeedback-assisted stress management training would augment parasympathetic nervous system activity, causing blood vessels to dilate and digital peripheral temperature to increase. This is consistent with the findings of Moser and colleagues in 1997. Moser showed that heart failure patients significantly increased their finger temperature from 91.5 ± 4.7°F to 94.6 ± 3.1°F after only one session of biofeedback combined with modified progressive muscle relaxation and imagery of hand warmth.[86]

We found that there was essentially no change in finger temperature following biofeedback training. Patients' average finger temperature was 89.9 ± 4.1°F before training and 89.8 ± 5.0°F after training. What is important to note is that while participating in this study, patients were still receiving standard medical management for their heart failure, and except for 3 patients whose medical records were unavailable (therefore we do not know what medications they were taking), all patients in the study were on some type of medication with vasodilatory properties. This was not the case in Moser's study, as medications were withheld from 12 hours before the study through the study duration.[86]

Heart Rate Variability

Autonomic imbalance is a hallmark of heart failure, reflecting a decreased resilience in the cardiovascular system to meet the demands of the environment, and is associated with increased morbidity and mortality.[11,60] In recent years, heart rate variability (HRV) has become a useful tool to measure autonomic balance, and depressed HRV has been shown to be a marker of poor prognosis in patients with heart failure.[90]

In this study, heart rate variability was measured by calculating the standard deviation of the inter-beat intervals (SDNN). This time domain measure is the most commonly used marker of autonomic function because it reflects all biological oscillations that lead to variations in heart rate.[110] On average, patients' SDNN significantly increased following biofeedback training, going from 32 ± 22 msec before biofeedback training to 44 ± 23 msec after biofeedback training.

The only other study showing the heart failure patients can use biofeedback to increase HRV came out in 2009 and showed that SDNN increased significantly following cardiorespiratory biofeedback training but only in patients with ejections fractions equal to or above 31%.[107] Disease severity was greater in our study (average LVEF 23 ± 15%), and it is now the first to show that end-stage heart failure patients with ejection fractions at or below 30% can increase their HRV following biofeedback training.

Furthermore, 25% of patients in the current study (5 out of 20) increased their SDNN from an unhealthy range (0-50 msec) to a moderately healthy range (50-100 msec) as defined by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology.[110] Movement into a higher category has been shown to increase a patient's probability of survival. In 1987, Kleiger and

colleagues found a 4-fold increase in the relative risk of death in 808 patients after myocardial infarction with low SDNN (≤ 50msec) compared to those with high SDNN (≥ 100 msec), and HRV remained the strongest predictor of death after accounting for demographics, medications, and various clinical factors.[60]

Psychophysiologic Reactivity and Recovery

In this study, instantaneous heart rate was analyzed before, during and after mental stress tasks in a psychophysiologic assessment before and after biofeedback training in an effort to measure both reactivity to and recovery from mental stress. Studies have shown that stress reactivity is associated with illness severity and is a predictor of later illness, especially cardiovascular disease.[63,114]

We expected that patients would react less to psychological stress following biofeedback training, and that this would be manifest as less of an increase in heart rate during mental stressors. Several studies have shown that biofeedback training can be used to control blood pressure during tests of mental stress[32,45,64,84], however we did not measure blood pressure in the current study. Using heart rate to quantify cardiovascular reactivity, we found no significant change following biofeedback training. We also did not show an increase in recovery from mental stress following biofeedback training. Instead no significant differences were found.

There are several limitations to the measurement of cardiovascular reactivity in the current study. First, the same stressors were used in the same order in both psychophysiologic assessments. Patients were informed prior to the last session that the second psychophysiologic assessment was going to be the same as they experienced in

the first session. It is difficult to know whether this knowledge would cause patients to be more relaxed in the second assessment because they know what is coming or if it could potentially cause patients to be more nervous, anticipating the stressors because they know what they are.

It is also necessary to consider the effect of the same team (biofeedback technician and therapist) administering the psychophysiologic assessment in both the first and last biofeedback sessions. It is possible that patients may have grown comfortable with the biofeedback team, and again, one could argue that this could make the patient more calm the second time around or it could add pressure to perform since it is clear to the patient that they should be less reactive following biofeedback training. Ruminating about performance in between tasks would certainly keep patients from recovering. One might also speculate that comfort with the biofeedback team could allow patients to be more vulnerable over time, actually responding more (reacting more) following biofeedback training. In this case, patients should still recover more quickly if they were successful with biofeedback training.

Homework

Patients were provided with relaxation CDs and handheld thermometers and asked to practice at least 20 minutes each day. Sheets were provided to record daily stress levels and as well as finger temperature before and after relaxation practice. These daily record sheets were collected at the beginning of each visit. One limitation to this approach is that digital peripheral temperature did not significantly change in the office, and therefore it is unclear if using this modality for home practice actually helped

patients. One might imagine that it was a source of frustration if patients felt unable to increase their finger temperature and therefore unable to succeed. On the other hand, some studies have shown that patients who practice a treatment do so because they find the treatment to be useful. Overall, 14 out of the 20 patients who finished all eight sessions of biofeedback (70%) completed some amount of homework.

Subjective Data

Patients were asked to self-report their level of relaxation after every activity (stressful and relaxing) in each psychophysiologic assessment. When stressors and relaxing activities were grouped together, patients reported being more relaxed during both types of activities after biofeedback training as compared to before training. One limitation to interpretation of this data is that patients knew they were in a study of relaxation and stress management, so perhaps they reported what was expected or what they felt was "the right answer." Without a validated measure of relaxation and a control group, there is no way to know if this response would be different in a group of heart failure patients who did not receive biofeedback-assisted stress management training or in any cohort based simply on test-retest effects.

Inpatients vs. Outpatients

With respect to respiration rate, inpatients made significant improvement after two biofeedback training sessions whereas outpatients required four training sessions in order to significantly lower their breathing rate. This difference may be due to the frequency with which patients were trained. Inpatients by definition were waiting for a

heart transplant in the hospital because they were the sickest of the patients on the transplant list and therefore the most likely to receive a heart transplant. These patients were seen more frequently (twice a week for four weeks) in order to have sufficient time to get through the entire biofeedback-assisted stress management training protocol before the patients came to transplant. Outpatients were seen once a week for eight weeks, and so perhaps even if they practiced on their own as encouraged, they would have benefitted more from more frequent respiratory biofeedback training with a certified psychologist present.

Across biofeedback sessions, outpatients showed a significant increase in SDNN that was not present in the inpatient sample. Although not a significant difference, this may be due to the fact that SDNN before biofeedback training was lower in the outpatient cohort relative to the inpatients. Because lower SDNN is a marker of poorer cardiovascular resilience and prognosis, this does not necessarily support the idea that inpatients are sicker than outpatients.

The same limitations exist with respect to cardiovascular reactivity and recovery when analyzed based on patient status, but the fact that inpatients had less time in between psychophysiologic assessments may have played a role in any differences between cohorts. Although not statistically significant, there seemed to be an inverse reactivity relationship between inpatients and outpatients before and after biofeedback training, wherein inpatients tended to react more following biofeedback training, and outpatients tended to react less. Perhaps inpatients remembered the stressors more than outpatients and anticipated them, thereby increasing reactivity.

In addition to the difference in the time course of training between inpatients and

outpatients, the treatment setting is also quite different. One might imagine that outpatients are living at home waiting for transplant and are therefore dealing with more real-life stressors than inpatients who are in a controlled hospital environment. As a result, perhaps they have more practice translating the relaxation and stress management skills learned in the study to everyday life such that laboratory mental stress tasks were not as arousing to this cohort after biofeedback-assisted stress management training.

Although they tended to react more, inpatients were shown to recover from mental stress significantly more than outpatients, and this did not change with biofeedback training. If taken as a measure of success with biofeedback, then greater cardiovascular recovery in the inpatient cohort might suggest that the inpatients were more successful than the outpatients. Again, the analysis of cardiovascular reactivity and recovery is complex, and it is possible that differences lie solely within the method of measurement and not a true difference.[70]

Surprisingly, with respect to the amount of homework completed, outpatients on average turned in more daily record sheets than inpatients. Assuming an equal probability that patients who did their homework would turn it in, this means that outpatients practiced more than inpatients. Because inpatients were living in the hospital, often with no visitors other then hospital staff, it was expected that they would practice more often just due to a lack of other things to do. Perhaps practice in between sessions was more relevant to this cohort because there was more time in between sessions. Of course the flaw in this approach is that is doesn't account for how many homework sheets each patient could have turned in. Perhaps looking at the percentage of homework turned in based on the total number of homework sheets the patient could have turned in would

reflect a different trend. Certainly the length of participation in the study varies between cohorts (inpatients are in the study for four less weeks), and therefore if an inpatient turned in the same number of daily record sheets as an outpatient, it would mean that the inpatient practiced more often.

4.2 The Effect of Biofeedback on Quality of Life

In this study, no significant changes in general or heart failure-specific quality of life (QOL) were observed following biofeedback training. When separated by patient status, however, some QOL differences did emerge. Keep in mind that in all cases, higher scores reflect a greater level of functioning.

On the SF-36, inpatients reported a lower social functioning score than outpatients, and this did not change with biofeedback. This makes sense because inpatients were waiting for a heart transplant in the hospital, and therefore probably had fewer opportunities to socialize than patients waiting for transplantation at home. Inpatients also reported a greater general health score as compared to outpatients, and this did not change with biofeedback. This may also be a result of inpatients living in the hospital because they have access to immediate care whenever they have a question, need a medication adjusted, etc.

With respect to the heart failure-specific Kansas City Cardiomyopathy Questionnaire, inpatients reported a lower quality of life score than outpatients which did not change with biofeedback training. Once again, this could be a result of inpatients living in the hospital while waiting for a heart transplant, perhaps missing the social connections and freedoms that come with living at home. On the KCCM, the clinical

summary score is an average of Physical Limitation Score and Total Symptom Score, reflecting how the patient is feeling physically with his or her heart failure. Inpatients reported higher clinical summary scores than outpatients, and this did not change with biofeedback. Again, one might imagine that access to around-the-clock medical care help inpatients manage the symptoms of heart failure better than outpatients living at home.

One limitation of the evaluation of quality of life in this study is the lack of an appropriate control group. We know that these patients' heart failure is progressing over time, and one might expect quality of life scores to decline as a result. Perhaps biofeedback kept heart failure patients in this study on an even keel such that we are actually underestimating the effect of biofeedback on quality of life.

4.3 The Effect of Biofeedback on Clinical Course

Clinical data were collected in outpatients only. Inpatients were unable to get out of bed to do the six minute walk test, and blood could not be drawn for plasma norepinephrine for logistical reasons.

Plasma norepinephrine was taken as a marker of sympathetic nervous system activity. High levels of circulating norepinephrine have become a biomarker of heart failure due to excess sympathetic input, and because we were teaching patients to decrease sympathetic activity, we expected plasma norepinephrine levels to decrease after biofeedback training. What we saw was that average plasma norepinephrine levels did not change following biofeedback training, however plasma norepinephrine did decrease in three of the eight patients.

Six-minute walk distance, a common measure of functional capacity in heart failure patients[3], also showed no significant change following biofeedback training. This finding is consistent with that of Swanson et al. who showed that exercise tolerance did not improve in patients with a left ventricular ejection fraction ≤ 30%.[107] The patients in the current study who completed the six-minute walk test all had ejection fractions at 30% or below. A study by Luskin in 2002 showed that a combination of biofeedback and stress management was associated with an increase in exercise tolerance (on average patients walked 175 feet further after the intervention), however again the patients in that study had New York Heart Association class I to "very early class III" heart failure, a population that has less severe disease relative to the patients in the Swanson and current studies.[73]

Even though average distance walked did not change with biofeedback training, the clinical cutoff for improvement or regression in functional capacity is 130 feet[3], and by this criterion, three patients showed clinical improvement in exercise tolerance. These were not the same three patients who showed decreased levels of circulating norepinephrine.

4.4 The Effect of Biofeedback on Myocardial Remodeling

In this study, we hypothesized that biofeedback could reverse the heart failure phenotype. We measured biological changes that we already know occur in heart failure and recover with LVAD support, including muscle contraction, beta-adrenergic and muscarinic receptor densities, as well as some of the calcium cycling proteins

downstream of these receptors. We hypothesized that biofeedback would also show recovery of such myocardial remodeling.

Muscle Function

Muscle function experiments were conducted in order to measure the response of individually dissected trabecular muscles to sympathetic nervous system stimulation. After finding the length at which each muscles produced its greatest contraction (Lmax), six contractile parameters were analyzed both at baseline as well as after a single dose of isoproterenol (ISO), a synthetic analogue of norepinephrine. All comparisons were made relative the non-failing group, and there were no significant differences in any of the six contractile parameters at baseline. This means that any differences we saw were due to our experimental manipulation (adding isoproterenol) and not to some initial difference in muscle function.

For all six contractile parameters, the response to isoproterenol (ISO) was measured as a percent change from baseline. Because it is known that sympathetic nervous system activity increases heart rate and force of contraction, we expected to see the following changes in the non-failing (NF) group: (1) Resting tension (RT) is the amount of tension a muscle generates at rest and was expected to decrease, (2) Developed tension (DT) is the amount of force a muscle generates during contraction and was expected in increase, (3) Time to peak tension (TPT) is the amount of time it takes for a muscle to reach the peak of contraction once it begins to contract and was expected to decrease, (4) Time to half relaxation (THR) is the amount of time it takes for a muscle to get from the peak of contraction to the halfway point of relaxation and was expected to

decrease, (5) Peak rate of tension rise (+dT/dt) is the point at which the muscle is contracting the fastest and was expected to increase, and (6) Peak rate of tension fall (-dT/dt) is the point at which the muscle is relaxing the fastest and was expected to increase. We know that these effects are depressed in muscles from patients with heart failure and to recover in muscles from patients with LVAD support. We hypothesized that the magnitude of the outlined changes will not be significantly different between the non-failing and failing + biofeedback (F + BF) groups.

The RT response to ISO decreased as expected with no significant differences among groups. Statistical significance was almost reached, however, and with mean values of -11.1 ± 6.0% and -10.6 ± 3.2%, the failing and F + LVAD groups showed less of a decrease in resting tension relative to the NF and F + BF groups (-16.1 ± 8.8% and -15.3 ± 2.9, respectively).

The DT response to ISO increased across all four groups, however muscles taken from failing hearts contracted significantly less than muscles taken from non-failing hearts. These results were as expected. In support of our hypothesis, there was no significant difference in DT response to ISO between the NF and F + BF groups, suggesting that biofeedback is associated with recovery of the functional response to ISO in individual cardiac muscles. On an individual patient basis, muscles taken from eight patients in the F + BF group contracted more than the average failing level, with muscles from three of these patients contracting at or above the non-failing level.

As expected, TPT and THR both decreased in response to ISO. No significant differences were found among groups with respect to either contractile parameter.

In order for heart rate to increase, the rate of contraction and relaxation must increase, and these results were shown in the peak rate of tension rise and fall responses to ISO across all four groups. As expected, the +dT/dt and -dT/dt responses were significantly lower in the failing group and recovered in the F + LVAD group. Although not to the same degree as the LVAD group, muscles in the F + BF group were not significantly different from muscles in the non-failing group with respect to +dT/dt or -dT/dt. For the three patients in the F + BF group whose developed tension response to ISO was at or above non-failing levels, the same was true of their +dT/dt and -dT/dt responses.

Beta-Adrenergic Receptors

Several studies have shown that beta-adrenergic receptor density is decreased in human heart failure, and that this reduction is reversed with LVAD support. In 2001, DiPaola and colleagues showed a 53% decrease in beta receptor density in failing human hearts relative to non-failing human hearts.[27] This confirmed the much earlier landmark study by Bristow and colleagues which also showed that there about 50% less beta receptors in failing human hearts than there are in non-failing human hearts.[19] Ogletree-Hughes et al. showed that beta-adrenergic receptor density in hearts with LVAD support was comparable to that in non-failing hearts.[92]

In the current study, we expected to replicate these results, showing a decrease in beta-adrenergic receptor density that is reversed in heart failure patients with an LVAD, and we hypothesized that the F + BF group would also show a recovery of beta-

adrenergic receptors, especially since we saw recovery in the functional response (developed tension response to ISO).

Although the magnitude of change was less (32%) than in previous studies (50% and 53%), we saw a significant decrease in beta-adrenergic receptor density in failing human hearts relative to non-failing hearts. We showed no significant difference in beta-receptor density between the non-failing and F + LVAD groups, confirming the recovery of LVAD-supported failing hearts shown in other studies. Beta receptor density in the F + BF group was significantly lower than that of the non-failing group, suggesting that biofeedback is not associated with the recovery of beta-adrenergic receptor density.

On an individual basis, three patients in the F + BF group did show recovery of beta-adrenergic receptors such that they were at or even above the non-failing average. Only one of these patients was one who also exhibited a developed tension response to isoproterenol at the non-failing level.

One can speculate that increased levels of circulating norepinephrine in heart failure patients may play a role in decrease in beta-adrenergic receptor density. If all of the excess NE were to bind the beta-receptors, the subsequent response would use up a ton of cellular ATP. Studies have shown normalization of plasma norepinephrine in LVAD patients, and so perhaps this is responsible for beta-adrenergic receptor recovery. This might also be a potential mechanism of action for biofeedback considering that some patients did exhibit beta receptor recovery. These patients were inpatients, however, and therefore plasma norepinephrine was not collected. In order to answer this mechanistic question, one would have to show a relationship between normalized (increased) beta receptor density and decreased plasma norepinephrine levels following

biofeedback training.

In receptor binding studies, the relationship between the rate of formation and dissociation of the ligand-receptor complex is called the equilibrium dissociation constant (K_d). K_d is the reciprocal of the affinity of a ligand for a receptor, and so K_d was measured across groups in this study as an inverse measure of binding affinity. This means that lower K_d values for a particular receptor indicate a higher binding affinity of the ligand for the receptor. One might imagine that functional changes can be due to an increased number of the receptor mediating the response or to an increased affinity of ligand for the receptor.

Results show no significant changes in beta-adrenergic receptor K_d across groups. In the F + BF group, however, half of the patients exhibited a much higher K_d than the other half of patients.

Muscarinic Receptors

Muscarinic receptor expression in the human heart is not nearly as well-characterized (in health or disease) as is the expression of beta-adrenergic receptors. Early studies of muscarinic receptor density in humans showed no significant differences in muscarinic receptor density between failing and non-failing hearts. In 1990, Bohm et al. measured muscarinic receptor density in 16 failing and 5 non-failing human hearts using radioligand binding with a tritiated antagonist and showed that although there were less receptors in failing hearts (211 ± 22 fmol/mg protein vs. 275 ± 21 fmol/mg protein in non-failing hearts) the difference was not statistically significant.[15] The opposite result was shown by Le Guludec and colleagues who used positron emission tomography (PET)

to non-invasively measure myocardial muscarinic receptors in 20 heart failure patients and 12 normal controls. This study showed that average muscarinic receptor density was significantly higher in the patients with heart failure relative to controls (34.5 ± 8.9 pmol/mL and 25.0 ± 7.8 pmol/mL, respectively).[65]

In the current study, we showed that muscarinic receptor density was significantly greater in failing human hearts relative to non-failing hearts (166.1 ± 45.9 fmol/mg protein vs. 92.1 ± 25.2 fmol/mg protein). LVAD-supported hearts exhibited even more muscarinic receptors, with a density of 221.1 ± 61.3 fmol/mg protein. Muscarinic receptor density in the F + BF group (123.2 ± 26.4 fmol/mg protein) was not significantly different from that of the non-failing group.

The only other study to measure muscarinic receptor density in heart failure patients with an LVAD came out of the Moravec laboratory last year, and it confirmed the results of the current study, showing a similar increasing relationship in muscarinic receptor density across non-failing, failing and LVAD-supported hearts.[42]

One might speculate that muscarinic receptors are up-regulated in failing hearts in an attempt to combat sympathetic nervous system (SNS) hyperactivity by increasing parasympathetic nervous system input to the cardiovascular system. Because the LVAD has been shown to reverse remodel the cellular and molecular changes associated with SNS hyperarousal, it would make sense to think that the need for elevated muscarinic receptors would go away, and there would be a normalization of muscarinic receptor density in LVAD-supported hearts. Instead, muscarinic receptor density was shown to further increase in the F + LVAD group. This may suggest that parasympathetic nervous system hypoactivation does not recover in patients with heart failure supported by an

LVAD. Because no significant difference was found in muscarinic receptor density between the non-failing and F + BF groups, this might also suggest that biofeedback is associated with a normalization of muscarinic receptors in patients with end-stage heart failure.

When the muscarinic receptor K_d was analyzed across groups, the F + LVAD group was significantly higher than the non-failing group. This means that binding affinity for muscarinic receptors is lower in LVAD-supported hearts. As previously discussed, the density of muscarinic receptors increased in this group. What is unknown is the temporal relationship between receptor density and receptor affinity. Perhaps muscarinic receptor density increased and then affinity decreased to compensate for an increase in signal transmission. Or maybe binding affinity for muscarinic receptors decreased and then receptor density increased in an attempt to restore the signal.

It is also possible that the differences in muscarinic receptor K_d are due to a difference in the distribution of muscarinic receptor subtypes, and it would be interesting to measure these subtypes across all four groups of patients going forward. While the exact reason for the difference in K_d in the F + LVAD group is unclear, our group of interest, the F + BF group, did not show a significant difference in muscarinic receptor binding affinity relative to non-failing hearts.

Calcium Cycling Proteins

With the exception of the ryanodine receptor, calcium cycling proteins were chosen because the changes in heart failure and heart failure with LVAD support were well-documented and well-understood. Specifically, SERCA is decreased and NCX is

increased in failing human hearts, leading to decreased contractile function. These changes in calcium cycling proteins are reversed and contractile function is improved in LVAD-supported hearts.[28] We expected to replicate these results in the current study, and we hypothesized that hearts in the F + BF group would exhibit reverse remodeling of calcium cycling proteins like what has been shown with LVAD support. In all cases, calsequestrin was used to normalize the data because protein levels have consistently been shown not to change in heart failure.[27,53,83]

Both SERCA/CALQ and NCX/CALQ protein levels showed no differences among groups in this study. The expected changes in failing and LVAD-supported hearts were not replicated, making comparisons between the non-failing group and F + BF group difficult to interpret. In a way, the lack of significant differences in SERCA/CALQ and NCX/CALQ protein levels between non-failing and F + BF hearts supports our hypothesis, however after consideration of the possible reasons why the expected changes were not replicated, it is unlikely that such a suggestion can be made.

What has been noticed in our laboratory is that calcium cycling protein measurements are variable based on the patient sample selected. Sometimes a group of patients exhibits the expected differences are shown, and sometimes they do not. This is worth exploring further since many gene therapy trials have begun to alter levels of calcium cycling proteins based on these "known changes" that we no longer see consistently in the laboratory.

The calcium cycling protein that did show changes across groups in this study was the ryanodine receptor. RYR/CALQ levels decreased significantly in the failing group relative to the non-failing group, and a further decrease was shown in the F +

LVAD and F + BF groups. Because there is no consensus in the literature with respect to RYR protein expression[51], the results of the current study are open to interpretation. If RYR protein levels are depressed in heart failure, this would mean less calcium would be released from the sarcoplasmic reticulum and into the cytosol to bind the myofilaments and cause cardiomyocyte contraction. The fact that RYR/CALQ levels do not recover in LVAD-supported hearts is counterintuitive, as one might imagine RYR receptors would be normalized in this group in order to improve muscle contractility.

Combined with the absence of changes in SERCA/CALQ and NCX/CALQ protein levels, a decrease in RYR/CALQ level does not support the normalization of contractile function that was shown in the F + BF group. Because it is a calcium release channel, decreased RYR protein would keep calcium from being released from the sarcoplasmic reticulum (SR). Without being released into the cytosol where it can bind to myofilaments and enhance myocardial contractility, calcium is of no use in the SR. Perhaps calcium is released through the IP_3 receptor which means that activation of the odd-numbered muscarinic receptors would be necessary. Without measuring muscarinic receptor subtypes, one can only speculate that the odd-numbered receptors play a greater role in releasing calcium into the cytosol and thereby improving contractile function in the F + BF group. The only other study that measured muscarinic receptor density in non-failing, failing and LVAD-supported hearts also measured muscarinic receptor subtypes and showed that the percentage of odd-numbered muscarinic receptors goes up in the F + LVAD group[42], supporting this idea as a possible mechanism in the current study for the F + BF group.

4.5 Correlation Data

In an attempt to determine whether patients who were successful with biofeedback were the same patients who showed biological changes in the heart, each biofeedback variable that changed was correlated with the four biological variables that changed or were variable including the developed tension response to ISO, beta-adrenergic receptor density, muscarinic receptor density, and RYR/CALQ protein concentration.

Although no significant relationships were found, two correlations included a data point that was outside the 95% confidence interval. This point was found to be from the same patient in both data sets, the only patient with congenital heart failure in this patient cohort. Because the mechanism of congenital heart failure may be different from dilated and ischemic cardiomyopathies, this point was removed from both data sets, and significant relationships emerged.

Patients who were breathing at slower rates in session eight of the biofeedback-assisted stress management training protocol showed greater expression of RYR protein levels. Even though overall RYR protein expression was lower in the F + BF group relative to the non-failing group, this suggests that success with respiration training may help to normalize RYR protein level.

The second significant correlation that emerged showed that patients who showed a greater percent decrease in respiration rate also showed the greatest developed tension response to isoproterenol. This suggests that breathing at lower rates may decrease background sympathetic nervous system arousal such that the cardiovascular system can respond to increases in sympathetic activity (demand).

The numbers of days patients waited between their last session of the biofeedback-

assisted stress management training protocol and their date of heart transplantation was also correlated with the four biological variables listed above, and no significant relationships were found.

4.6 Summary

With improvements like a decrease in respiration rate and an increase in heart rate variability, end-stage heart failure patients in this study were certainly able to use biofeedback to learn how to modify certain physiologic variables. Although no control group was included for comparison, it is possible that biofeedback-assisted stress management training may have improved quality of life and clinical course in some patients in the cohort. Many patients asked to continue receiving biofeedback after their participation in the study had ended, and sometimes cardiologists requested that we enroll one of their patients in the study because they felt it would be beneficial to them. One patient's medical chart documents her comments specifically regarding participation in this biofeedback study. She says, "I thought it was helpful. When going into an anxiety state, I can get into a calm place. It helps with negative thoughts." When asked whether or not biofeedback has lost its effectiveness five months after the study, she said no.

On the biological side, contractile response to beta-adrenergic stimulation recovered in some patients, and other patients showed recovery in beta-adrenergic receptor density. Because the expected results with respect to calcium cycling proteins were not exhibited, it is unclear what the lack of differences between SERCA and NCX expression in NF and F + BF hearts actually means. RYR protein expression was shown to be decreased in the F + BF group which may have something to do with the recovery of muscarinic receptor

density also shown in this group, however further studies must be done in order to explore the mechanisms involved in the biological changes shown in this study.

4.7 Future Directions

One question that was left unanswered in this study is whether or not patients incorporated the relaxation and stress management skills into their daily lives. It is possible that patients were able to control their physiology when prompted to do so during biofeedback training, and a follow-up measurement at some later date after the conclusion of the study would provide some insight into the endurance of biofeedback-assisted stress management training.

Considering the recent work by Kevin Tracey that showed decreased vagal control of the heart can have pro-inflammatory consequences that exacerbate the heart failure condition[57,113], it might also be interesting to measure pro-inflammatory cytokines before and after biofeedback training. Other questions one could ask are (1) Does biofeedback decrease transplant recovery time? or (2) Does biofeedback decrease transplant rejection? In order to measure parasympathetic nervous system activity to correlate with these endpoints would be to use frequency domain analyses of heart rate variability.

Over the course of this study, it has become clear that the end-stage heart failure population is very challenging to study, and it would be interesting to see if biofeedback-assisted stress management training can be effective in patients with earlier stages of cardiovascular disease. Gender and diagnosis-specific differences may also be worth exploring in the future as the potential for biofeedback-assisted stress management training to change cardiovascular biology continues to grow.

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